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CONTROLLING FACTORS IN METHANE FERMENTATION

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Summary of Research Progress

Controlling Factors in Methane Fermentation
U.S. Public Health Grant WP-00394
Period Covering 9-1-62 to 9-1-64

R. E. Speece, Principal Investigator
R. S. Engelbrecht, Co-Principal Investigator

INTRODUCTION

A number of approaches have been taken in attempting to evaluate the important factors which control the rate of methane fermentation in anaerobic digestion. The ultimate goal of this research has been to determine the chemical, physical, and biochemical requirements which will enable the methane fermentation to proceed at rates on the order of one magnitude greater that commonly observed in conventional anaerobic digestion.

A considerably detailed study was conducted to assay the methane fermentation stimulation potential of six compounds which had shown promise in a previous study conducted by the principal investigator. Next, a series of studies was designed to evaluate the influence of many physical and chemical factors involved in digestion. Following this, the methane fermentation requirements for trace organics, such as vitamins and amino acids was studied. Combinations of individual amino acids and vitamins as well as mixtures, e.g. casein hydrolyzate (enzymatic), yeast extract, and fortified vitamin B complex, were assayed for their stimulation potential. The surface charge on the microorganisms was altered by inorganic and organic coagulating agents while observing the acetate utilization rate.

Additional fractionation of complex stimulatory substances such as sewage sludge digester supernatent and rumen fluid is necessary in order to more definitely indentify the combination of nutrient requirements which enable methane fermentation to proceed high rates. Some fractionation work was done several years ago at M.I.T. and additional studies are presently underway on this project. In work done with methane organisms in the field of Dairy Science, Bryant has found a requirement for the higher branched and normal fatty acids, such as, methylbutyric, isobutyric, n-valeric and n-butyric. These were obtained from the acidified ether extract fraction. To date, these acids have shown no stimulatory effect on the mixed cultures under investigation, but further fractionation is underway.

Cation and anion exchange, solvent extraction, activated carbon adsorption, distillation, chromatographic separation, acid and base hydrolysis, and other fractionation procedures are presently being used both singularly and in combination to more clearly identify the stimulatory agent or agents. Assay for stimulation potential is being conducted with sludge taken directly from a sewage sludge digester as well as an enriched culture taken from a "washed out" stock digester.

Reprints of an article on the "Response of Anaerobic Digestion to Frequent Temperature Variation", are included as part of this progress report.

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Evaluation of Stimulation Potential of Supposed Stimulants - Part I

The initial phases of the study were designed to evaluate the effects of a number of compounds which had produced stimulation in a previous study. These compounds were: iron, cobalt, thiamine, proline, glycine, and benzimidazole. A series of eight 1-liter digesters was set up from a large washed out stock digester which was purged of the original sludge by daily wasting of the contents and replacement by a feed which consisted of acetate, inorganic nutrients and tap water.

The digesters were fed a combination of the compounds according to the following chart:

Digester No.	Fe	Co	Thiamine	Proline	Glycine	Benzimidazole
1	-	X	X	X	X	X
2	X	-	X	X	X	X
3	X	X	-	X	X	X
4	X	X	X	-	X	X
5	X	X	X	X	-	X
6	X	X	X	X	X	-
7	X	X	X	X	X	X
8	-	-	-	-	-	-

The concentration of each compound was fed so as to maintain a concentration of 20 mg/l in the digester. Digesters 7 and 8 served as controls in which all of the compounds and none of the compounds were fed respectively. The substrate was maintained at approximately 1500 mg/l as acetic acid and fed in the form of the neutral salt calcium acetate. The digesters were operated on a 30-day detention time. The pH was maintained near neutrality by maintaining the alkalinity at 3000 mg/l as calcium carbonate by the addition of $NaHCO_3$. Additional inorganic salt concentrations in the digester were maintained as follows:

266 mg/l $(NH_4)_2$ HPO_4
27 mg/l KCL
66 mg/l $MgSO_4$

Results of First 80 Days of Operation

This set of digesters was then operated according to the above schedule for 80 days and the results are shown in Figure 1. The digester which received all of the assay compounds except iron showed a low rate of acetate utilization, 200 mg/l/day, and progressively decreased to about 100 mg/l/day. The digester which received all assay compounds except cobalt exhibited an acetate utilization rate of 200 mg/l/day initially, but rose to approximately 1000 mg/l/day. The digester receiving no thiamine was retarded for the first 30 days and utilized only about 200 mg/l/day of acetate, but then the utilization rate rose to approximately 600 mg/l/day. The digester receiving no proline increased from 200 to a maximum of 700 mg/l/day. The utilization rate in the digester receiving no glycine increased steadily from 200 to about 700 mg/l/day. The digester receiving no benzimidazole responded similarly to the digester receiving no proline. The control digester receiving all assay compounds responded much the same as the digester receiving no proline. The control digester

receiving none of the assay compounds rose from 200 to 500 mg/l/day in the first 45 days and then declined to 300 mg/l/day in the last phase.

The results of this study for 80 days showed a markedly lower acetate utilization for the two digesters receiving no iron and a significantly higher rate for the digester receiving no cobalt. The other digesters operated at about the same rate in a zone between these two extremes.

Results of Operation From Day 81 to 170

Figure 2 shows graphically the digestion rates for the period Day 81 to 170. Operational changes were made on Day 81 and Day 90. On Day 81, additions of 5 mg/l of iron were daily added to the digester which received all assay compounds except iron. On Day 90 three changes were made: the concentration of cobalt was reduced from 20 to 1 mg/l in all digesters which received this compound, and the hydraulic detention time in all digesters was reduced from 30 to 10 days. Also, all of the assay compounds were reduced to 1/3 their original concentration until Day 140 when they were again added at the original concentration. The following table shows the concentration of compounds added to the respective digester feeds, expressed in mg/l:

Digester No.	Fe	Co	Thiamine	Proline	Glycine	Benzimidazole
1	1.67	6.7	6.7	6.7	6.7	6.7
2	1	-	1	1	1	1
3	6.7	6.7	-	6.7	6.7	6.7
4	6.7	6.7	6.7	-	6.7	6.7
5	6.7	6.7	6.7	6.7	-	6.7
6	6.7	6.7	6.7	6.7	6.7	-
7	6.7	6.7	6.7	6.7	6.7	6.7
8	-	-	-	-	-	-

A very marked increase in acetate utilization was evident when iron was added to the digester which previously received all assay compounds except iron. The rate increased from 200 to 720 mg/l/day in the period from Day 80 to 100. The digester receiving none of the assay compounds decreased progressively to nil utilization by Day 130 and was discarded. From Day 140, when the concentration of assay compounds was tripled back to the original level, the digester receiving all compounds operated at a slightly higher rate than the other digesters.

Generally speaking, during this period, all digesters except the one receiving none of the assay compounds, operated at approximately the same utilization rate.

Results of Operation from Day 170 to 255

Figure 3 illustrates the results during this period. Since the acetate utilization rate in all of these digesters was limited to approximately 400 mg/l/day, an attempt was made to change the conditions so that higher rates could be achieved.

Therefore, the nutrient salts added along with the feed were altered. Amonium bicarbonate was substituted for sodium bicarbonate as the principal buffer. This increased the ammonium nitrogen content in the digesters from about 60 to 800 mg/l as nitrogen. From Day 200, the concentration of the basic mono- and di-valent cations was increased.

There was no noticeable effect in the substitution of ammonium bicarbonate for sodium bicarbonate and the consequent increase in ammonium nitrogen concentration. A minor increase from approximately 300 to 500 mg/l/day of acetate utilization was noted when the mono and divalent cations concentration was increased. However, during this entire study of 255 days, one or more limiting conditions existed in all digesters which prevented the achievement of significantly high rates of acetate utilization.

Evaluation of Stimulation Potential of Supposed Stimulants - Part II

Eighteen digesters were inoculated with 500 ml of seed drom a "washed out" digester and diluted to one and a half liter with buffered nutrient water. The stimulation potential of the compounds which had produced stimulation in a previous study were assayed individually and in combinations. These digesters were operated for 170 days.

Period I

From Day 1 to 75, the digesters were operated on a 33 day D.T. with the nutrient salt concentration in the feed maintained as follows:

$MgSO_4$ - 140 mg/l
K Cl - 30 mg/l
$NH_4H_2PO_4$ - 300 mg/l
$NaHCO_3$ - 5000 mg/l

The digesters were fed a combination of compounds at 20 mg/l each during Period I according to the following schedule.

Digester	Fe^{+++}	Co^{++}	Thiamine	Proline	Glycine	Benzimidayole	Al^{+++}
A Control	-	-	-	-	-	-	-
B Control	-	-	-	-	-	-	-
C	X	-	-	-	-	-	-
D	-	X	-	-	-	-	-
E	-	-	X	-	-	-	-
F	-	-	-	X	-	-	-
G	-	-	-	-	X	-	-
H	-	-	-	-	-	X	-
I Control	-	-	-	-	-	-	-
J	X	X	-	-	-	-	-
K	-	X	X	-	-	-	-
L	-	X	X	-	-	X	-
M	-	X	X	-	X	X	-
N	-	X	X	X	-	X	-
O Control	-	-	-	-	-	-	-
P	-	X	X	-	X	-	-
Q	-	X	X	X	-	-	-
R	-	-	-	-	-	-	X

Period II

From Day 75 to 95, the detention time was reduced from 33 to 23 days. The concentration of assay compounds and nutrient salts in the feed was maintained the same as during Period I.

Period III

From Day 95 to 125, the detention time was reduced from 23 to 10 days. The concentration of assay compounds and nutrient salts in the feed was maintained the same as during Period I.

Period IV

From Day 125 to 170 the iron concentration in digesters C and J was reduced from 20 to 5 mg/l. Also 5 mg/l of iron was added to the feed of the remaining digesters with the exception of the four control digesters A, B, I and O. The concentration of cobalt was reduced from 20 to 1 mg/l in the digesters which were scheduled to receive a cobalt in the feed. The detention time remained at 10 days.

The following tabulation shows the rate of acetate utilization during the respective periods:

Acetate Utilization Rate - mg/l/days

Digester	Period I	Period II	Period III	Period IV
A	206	261	248	198
B	230	284	248	179
C	309	337	284	183
D	117	56	103	121
E	173	213	222	145
F	193	273	231	164
G	233	249	211	169
H	187	143	200	185
I	186	229	233	211
J	198	340	383	358
K	146	210	118	104
L	83	77	118	93
M	47	17	103	97
N	69	32	115	102
O	199	197	268	225
P	80	100	101	94
Q	165	150	95	190
R	234	254	237	185

These results are plotted in Figure 4 on a bar graph showing the respective assay compounds in each digester.

Discussion

The significant effects during the operation of each digester are noted below:

Digester C operated significantly higher than the control during the Period I. However, during Period II and III, it operated only slightly higher than the control and operated comparable to the control during Period IV.

Digester D operated consistently below the control. The change during Period IV increased the rate to about comparable with the control.

Digester E decreased to a rate below the control during Period IV.

Digesters F, G, and H operated at or slightly below the control during all four periods. Addition of iron in Period IV had negligible effect.

Digester J operated comparable to the control during Period I and significantly higher during Period II. A marked increase in rate above the control occurred during Period III when the detention time was cut to 10 days. However, when the iron was reduced from 20 to 5 mg/l and the colbalt was reduced from 20 to 1 mg/l, the rate fell to that of the control.

Digester K operated generally below the control. Very little change was noted during Period IV.

Digester L operated significantly below the control with very little change during Period IV.

Digester M operated at almost a NIL rate during Periods I and II, and then increased to about 50% of the control rate during Period IV. There was no apparent effect during Period IV.

Digester N operated significantly below the control during Periods I and II, but began to approach the control rate during Period III. There was no apparent effect during Period IV.

Digester P operated significantly below the control during all four periods.

Digester Q operated slightly below the control during Period I, while the rate dropped even lower during Period II. Period III resulted in an almost NIL rate, but by adding the 5 mg/l of iron and reducing the cobalt from 20 to 1 mg/l in Period IV, there was a steady increase in rate until it exceeded the control.

Digesters Q and R operated comparable to the control during all periods.

The stimulatory effect of iron is apparent as noted by the acetate utilization rates in Digester C during Periods I, II, and III when it was added at a concentration of 20 mg/l in the feed. The other obvious stimulation occurred in Digester J during Periods II, III, and IV. This digester received a combination of iron and cobalt.

The inhibitory effect of 20 mg/l of cobalt in the feed is marked during Periods I, II and III in all the digesters which received cobalt except Digester J which also received iron. Many of these digesters operated at less than 50% of that of the control.

The addition of thiamine, proline, glycine, and benzimidazole slightly retarded the rate of acetate utilization when added singularly to a digester. It appears the addition of thiamine overcame the toxic effect of cobalt when added in this combination in Digester K as compared to Digester D during Period II. Proline was slightly more stimulatory than glycine.

Effect of Inert Surface on Acetate Utilization Rate

Two replicate digesters were set up to evaluate the stimulatory effect on acetate utilization of finely ground asbestos. One digester received 30 grams per liter of finely ground asbestos on the first day only while the second served as a control and received none. The seed used to inoculate both digesters come from a "washed out" stock digester being fed calcium acetate. The feed to both digesters contained the same concentrations of nutrient ions Na, NH_4, K, PO_4, Mg and sufficient acetate to maintain between 500 and 1500 mg/l of volotile acids in the digester at all times. Both digesters were operated on a 10 day detention time and mixed once per day. The duration of this study was 40 days.

Results

For the first 10 days, both digesters operated at the same rate. Thereafter until the end of the study, the rate of utilization in the digester which received asbestos operated at a rate approximately double that of the control digester as shown in the accompanying graph. The digestion rate in the control digester fell from about 700 to 350 mg/l/day by the end of the run, while the digester with asbestos operated at a rate between 900 and 1000 mg/l/day during the period. These results are shown in the Figure 5.

It should be noted that it was not possible to withdraw a uniform sample of the contents from the digester containing asbestos. This may have resulted in less wastage of cell mass from this unit as compared to the control and accounted for part of the increased digestion rate.

Effect of Asbestos, Asbestos Extract, 20 mg/l Iron, and No Additive on Acetate Utilization Rate

Since the presence of asbestos in a digester resulted in increased acetate utilization, an attempt was made to evaluate more closely the phenomena involved. Asbestos contains certain minerals such as calcium, magnesium, iron and manganese. It was hypthesized that the stimulatory effect of asbestos resulted from the extraction of required minerals from the asbestos under anaerobic conditions. Therefore, four replicate digesters were set up from a stock "washed out" digester. One digester received only substrate and nutrient salts and tap water with no additives in the feed solution and thus served as a control. The second digester received 20 gm/l of asbestos on the first day. The third digester received the anaerobic extract from asbestos, instead of tap water in the daily feed solution while the fourth digester received 20 mg/l of iron in the daily feed.

These digesters were operated for 35 days and the results are shown in Figure 6. It is evident that the digester containing asbestos yielded the highest rate of acetate utilization. This would indicate that the function of asbestos is not just a reservoir of iron or other essential minerals which can be leached out under anaerobic conditions. Something in addition, surface area being one factor, is provided by the presence of asbestos.

Effect of $CaCO_3$ Solids on Acetate Utilization Rate

References are found in the literature of bacteria which multiply more rapidly in the presence of a material which increases the surface area, such as beads or an inert, finely divided precipitate. The response of the methane-forming organisms was evaluated in the presence of 0, 1, 5, 10 and 20 grams/liter of $CaCO_3$.

Five replicate 750 ml digesters were set up from a "washed-out" stock digester. The above concentrations of $CaCO_3$ were added initially and sufficient amounts were present in the daily feed to replenish the inert solids withdrawn daily. Stock nutrients were continually supplied and the acetic acid substrate was maintained at 1000 to 1500 mg/l.

Results

Figure 7 indicates that the digesters containing the higher concentrations of $CaCO_3$ operated at a higher rate of acetate utilization, especially during the period of Days 15 to 35. Some adverse effect occurred in the digester containing 5 gm/l which drastically decreased the rate between Days 6 and 14. However, it recovered from this by the end of the study. Also, air leakage on Day 27 in the digester containing 20 gm/l temporarily reduced the rate in that digester.

The mechanism whereby the presence of $CaCO_3$ solids resulted in higher acetate utilization rates is not apparent.

Effect of Volatile Acids Concentration on Acetate Utilization Rate

Two replicate digesters were set up to observe the effect of acetate concentration on utilization rate. Both digesters were initially purged of oxygen with a gas mixture of 50% carbon dioxide and 50% nitrogen, and were then charged with 750 ml of seed from a "washed out" stock digester which was fed calcium acetate as the substrate and nutrient salts containing ammonia, phosphate, potassium and magnesium. Iron was initially added to both digesters at the rate of 50 mg/l. No buffer was fed, since the neutral calcium salt of acetic acid was fed as the substrate.

Both digesters were operated identically for three weeks to establish a common baseline of operation. After it was established that they were operating at comparable utilization rates, the volatile acids concentration in one digester was maintained at approximately 1300 mg/l, while the volatile acids concentration in the second digester was maintained at approximately 3500 mg/l. Both digesters were then operated for 60 days.

Results

The operational results are shown in the following table:

Period of Operation	Digester I		Digester II	
	Ave.V.A.conc. (mg/l)	Ave.Utilization Rate (mg/l/day)	Ave.V.A. conc. (mg/l)	Ave. Utilization Rate (mg/l/day)
Days 1-30	1360	320	3780	390
Days 31-60	1230	190	3250	540
Days 1-60	1290	250	3530	460

It is noted from the above table that during the first month of operation Digester II operated at about 120% of the utilization rate of Digester II. However, during the second month, the utilization rate in Digester II was 280% of that in Digester I. These rates are based on gas production values and therefore are corrected for the higher amount of volatile acids contained in the daily samples withdrawn from Digester II which contained the higher concentration of volatile acids.

It appears from this that the microorganisms are dependent on substrate concentration and that the reaction is not of zero order. The higher concentration of volatile acids appears to be responsible for the increase in utilization rate from 390 to 540 mg/l/day in Digester II during the second month as compared to a simultaneous decrease in rate from 320 to 190 mg/l/day in Digester I. Two factors remain unevaluated in this study: first, the effect of additional precipitated calcium bicarbonate and carbonate in Digester II due to the higher feed rate of calcium bicarbonate, and second, the effect of adding 50 mg/l of iron initially, but not replenishing it in the daily feed. The latter was an oversight in this study. It should have been added daily.

Study of the Effect of Detention Time on Acetate Utilization Rate

Two replicate digesters were set up from a "washed out" stock digester. One digester was operated on a 6 day detention time while the second was operated on a100 day detention time. The same concentration of nutrient salts was maintained in the feed for both digesters. These digesters were operated over a 45 day period.

Results

The acetate utilization rate at 6 days D.T. averaged 208 mg/l/day, while at 100 days D.T., it averaged 555 mg/l/day. Thus, the overall utilization rate was 2.7 times faster at the longer detention time. However, the organic nitrogen concentration, which is taken as a measure of the bacterial cell mass, averaged approximately 7.0 mg/l in the 6 day D.T. digester and 60 mg/l in the 100 day D.T. digester. Using a factor of 9.4 to convert from organic nitrogen to cell mass, this yields 66 mg/l of cells in the 6 day D.T. digester and 560 mg/l of cells in the 100 day D.T. digester.

Thus, the acetate utilization rate per gram of cells is as follows:

$$\frac{208 \text{ mg/l/day}}{66 \text{ mg/l of cells}} = 3.15 \frac{\text{gm acetate/day}}{\text{gm cells}} \text{ @ 6 day D.T.}$$

$$\frac{555 \text{ mg/l/day}}{560 \text{ mg/l of cells}} = 1.0 \frac{\text{gm acetate/day}}{\text{gm cells}} \text{ @ 100 day D.T.}$$

This indicates that a unit mass of cells in the 6 day D.T. digester utilized approximately 3 times as much substrate as a unit mass of cells in the 100 day D.T. digester.

The net synthesis of substrate into cell mass was as follows:

6 day D.T. digester:

66 mg/l cells x 1/6 wasting = 11 mg/l cells synthesized/day

$$\frac{11 \text{ mg cells synthesized/day}}{209 \text{ mg acetage utilized/day}} = 5.3\% \text{ synthesis of acetate into cell material}$$

100 day D.T. digester:

560 mg/l cells x 1/100 wasting = 5.6 mg/l cells wasted/day

$$\frac{5.6 \text{ mg cells wasted/day}}{555 \text{ mg acetage utilized day}} = 1.0\% \text{ synthesis of acetate into cell material}$$

Effect of Mixing on Acetate Utilization Rate

A study was made of the effect of mixing on the rate of acetate utilization. Two digesters were set up and operated identically, with one digester being mixed continuously and the other being mixed only once per day at the time of feeding. Both digesters were operated on a 10 day hydraulic detention time and received the usual inorganic nutrients in the feed. Volatile acid concentration was maintained so that a surplus was available except for a number of isolated instances in which the level dropped to less than 500 mg/l. The digesters were simultaneously operated for 90 days.

Results

The digester which was continuously mixed utilized acetate at approximately 2.8 times the rate of the non-mixed digester. The volatile acid utilization rates are shown in the following table for comparison:

	Acetate Utilization Rates mg/l/day		
	Average	Maximum	Minimum
Continuously mixed digester	486	720	190
Non-mixed digester	173	450	20

Figure 8 is a graphical comparison of the digester performances.

Assay of Trace Organics for Stimulation of Acetate Utilization Rate

A series of digesters were set up with 100% well digested sludge from the Champaign-Urbana sewage treatment plant. Trace amounts of various organic compounds, reported to promote the growth of various anaerobic microorganisms, were added on the first day to the digesters in the following combinations:

Digester 1:

Calcium Pantothenate	- trace
Adenine	- "
Pyruvate	- "
Thioctic acid	- "
Thiamine	- "
Nicotinamide	- "
L-Cystine	- "
B-Alanine	- "
Benzoate	- "

Digester 2:

Calcium Pantothenate	- 10 mg/l
Pyruvate	- 10 mg/l
Adenine	- 10 mg/l

Digester 3:

Menadione	- 10 mg/l
Calcium Pantothenate	- 10 mg/l
Vitamin B_{12}	- trace
Biotin	- trace

Digester 4:

Adenine	- 10 mg/l
Thiamine	- 10 mg/l
Calcium Pantothenate	- 10 mg/l
Nicotinamide	- 10 mg/l
Thioctic acid	- trace

Sufficient acetic acid was fed daily to maintain the volatile acids concentration above 500 mg/l so that excess substrate was present at all times. Inorganic nutrients were also included in the daily feed. The addition of a fortified vitamin B complex failed to produce stimulation also.

None of these four combinations of trace organic materials was able to produce a noticeable stimulation in the rate of acetate utilization when added to digesters containing 100% well digested sludge. Nor did the addition of a fortified vitamin B complex produce stimulation either. This indicates that in a sewage sludge digester, these trace organic compounds are not the rate limiting factor in acetate utilization.

Effect of: Surface Charge - EDTA - Hay ash - $K_3Fe(CN)_6$ - KCN - Egg Albumin

Surface Charge - $AlCl_3$

A series of digesters was set up to observe the effect of surface charge of the bacteria on the rate of acetate utilization. Five digesters were anaerobically charged from a stock digester which had been purged of the original seed sludge. Acetate and nutrient salts were fed daily to provide substrate and replace the nutrients withdrawn in the daily sample. Aluminum chloride was fed to the respective digesters in the following concentrations: 0, 50, 100, 200, and 400 mg/l.

Results

There was little difference in the acetate utilization of the five digesters. The digester which received 200 mg/l $AlCl_3$ operated during two periods at a rate above the control, but generally operated comparable to the control. The digesters receiving 50, 100, and 400 mg/l $AlCl_3$ operated equal to or less than the control. Figure 9 illustrates the comparative rates. This preliminary study indicates that the effect of making the bacterial surface charge more positive does not appreciably increase the rate at which the anionic acetate substrate is utilized. It does indicate that the addition of a coagulant for removal and recycle of bacterial mass in a waste stream is feasible, since the solids settled very rapidly, leaving a clear supernatent without appreciably diminishing the rate of digestion.

Surface Charge - Cationic Polyelectrolyte

A cationic polyelectrolyte was added to a digester which had been purged of essentially all of the original seed sludge. Traces of this coagulant were added on Days 1, 7, and 14. From Days 20 to 33, it was added along with the daily feed.

Results

Figure 10 illustrates there was no significant stimulation or repression of acetate stimulation due to the polyelectrolyte, except after about 10 days of daily addition when the rate gradually started to decrease. The digester had been continually agitated on a shaking table, but as soon as the shaking was stopped, the solids immediately dropped to the bottom, forming a dense sludge layer. The supernatent appeared comparable to tap water by visual comparison. Again, this preliminary study indicated that bacterial surface charge had little effect on acetate utilization.

Complexing Agent-EDTA

The addition of EDTA to a digester, purged of the original seed sludge, was observed and the result is shown in Figure 11. A slug addition of 100 mg/l was made on Day 14 and daily additions were started on Day 20. The following graph shows the acetate utilization rate vs time. It is difficult to draw any conclusions from this preliminary study. The effect of EDTA on the acetate utilization rate is questionable. There was no significant effect after the slug addition of EDTA on Day 14 and an initial decrease followed by a subsequent increase in rate after daily additions of EDTA were commenced. It should be pointed out that this study indicated primarily the effect of EDTA itself on the microorganisms. There would be little opportunity for the complexing action to become evident, since any materials which had precipitated from solution would not be returned to solution after the EDTA addition, and thus complexed. The EDTA should be added before any critical materials have been removed from solution.

A study is presently in progress in which EDTA was added prior to the mixing of the nutrient salts and innoculation with seed. The objective of this is to determine the beneficial or detrimental effect of a complexing agent in complexing toxic ions or prevent precipitation of required ions.

Effect of Alfalfa Hay Ash

Since methane organisms are found in the rumen of cattle, the beneficial effect of minerals found in alfalfa hay was assayed. Fifty grams of hay was ashed and added to a digester on two occasions. No significant stimulation in acetate utilization occurred.

Effect of Egg Albumin

The effect of a complex, water soluble protein was determined by adding egg albumin to a digester. The purpose of this was to pick a protein with a large number of charged sites which were thus capable of altering the charge on the microorganisms. There was no significant effect on the rate of gas production.

Effect of Cyanide

Vitamin B_{12} has been found to be present in anaerobic digestion by a number of workers.[12] Since a cyanide ion is incorporated in the molecular structure of vitamin B_{12}, the effect of adding small amounts of cyanide to a digester was evaluated. One very insoluble form of cyanide was added, $K_3Fe(CN)_6$ and another more soluble form KCN was added in another instance. Toxicity resulted from the addition of 10 mg/l KCN while no significant changes in acetate utilization were apparent after adding 10 mg/l $K_3Fe(CN)_6$.

SUMMARY

A number of factors have been elucidated which stimulated the rate of methane fermentation. Among these were:

Iron alone.
Iron and cobalt in combination..
Asbestos.
Calcium carbonate solids.
Increased volatile acids concentration.
Increased sludge retention time.
Continuous mixing.
Increased temperature to an upper limit of 45°C.

However, the acetate utilization rates achieved have been low, with a maximum of 2500 mg/l/day for both acetic acid and propionic acid substrates. These maximum rates were achieved with a seed sludge taken directly from a sewage sludge digester, while the rates for seed taken from "washed-out" stock digesters were less than 1000 mg/l/day. Thus far, all of the necessary combinations of physical, chemical, and biochemical factors which will produce high rates of acetate utilization have not been determined.

It is anticipated that the further fractionation of the complex stimulatory materials will more clearly identify the unknown factors. This, coupled with procedures developed in the field of Dairy Science for determining growth requirements of pure culture methane-forming organisms holds considerable promise of providing a fruitful approach to this exceptionally significant problem.

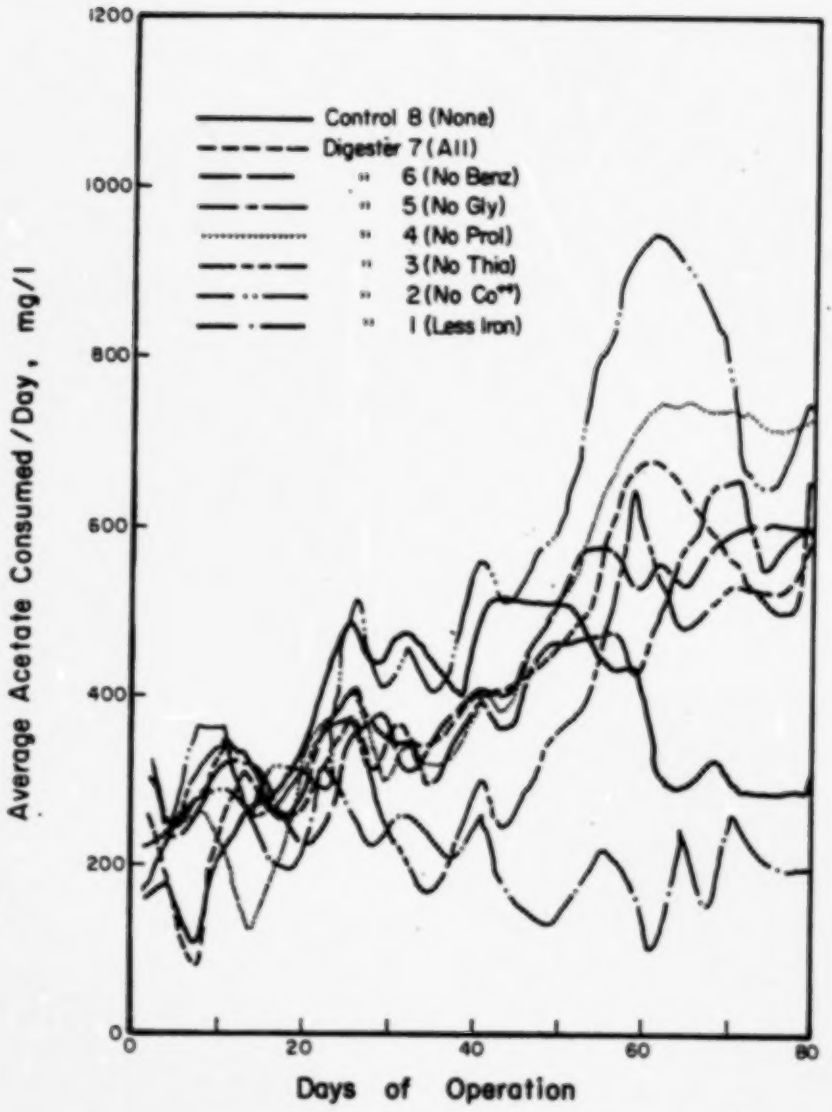

FIGURE I. COMPARATIVE EFFECT OF ADDITIVES - DAY 1 TO 80.

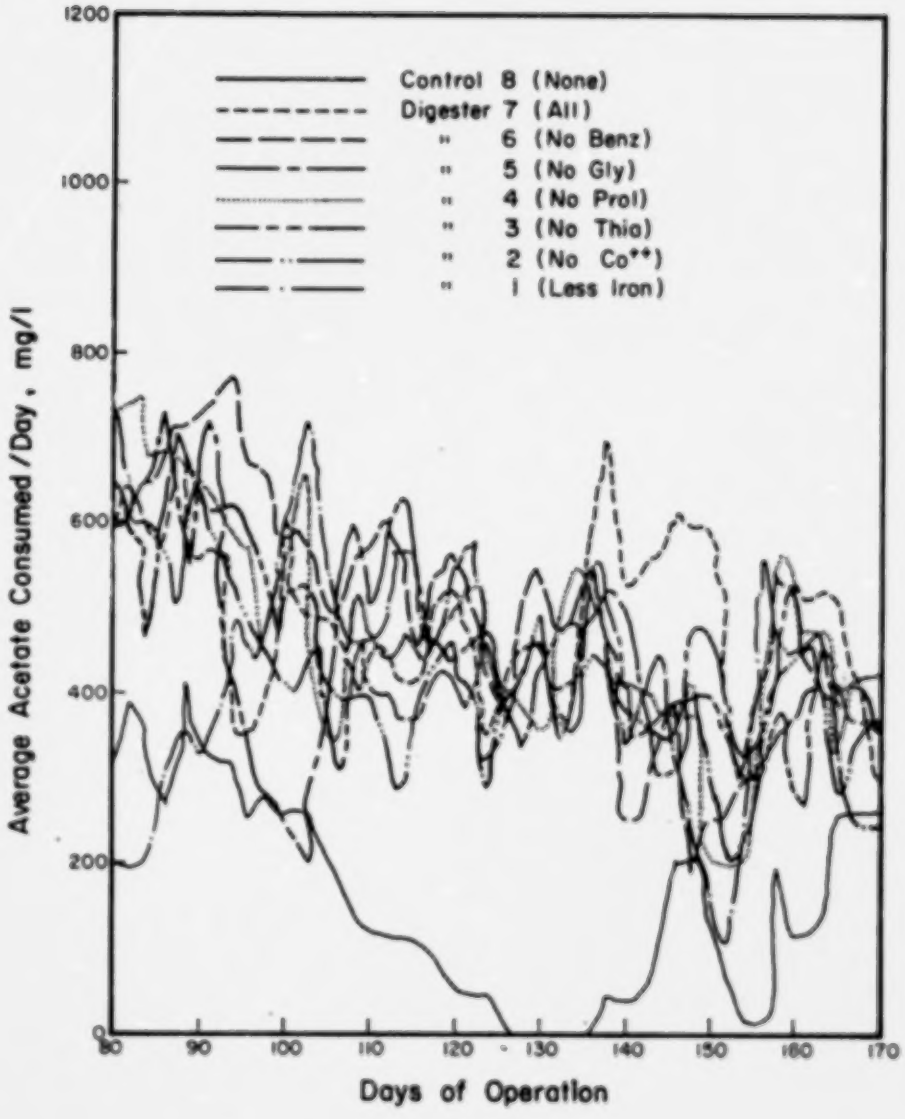

FIGURE 2. COMPARATIVE EFFECT OF ADDITIVES — DAY 81 TO 170.

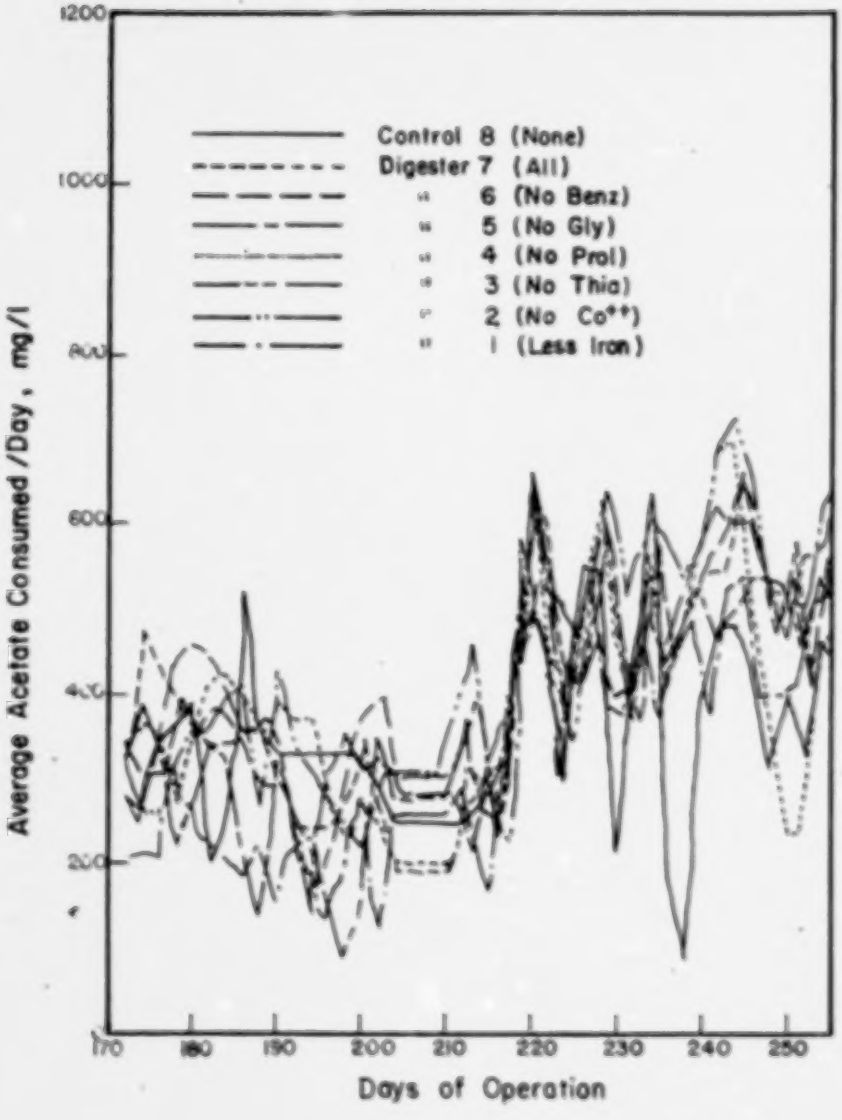

FIGURE 3. COMPARATIVE EFFECT OF ADDITIVES— DAY 171 TO 250.

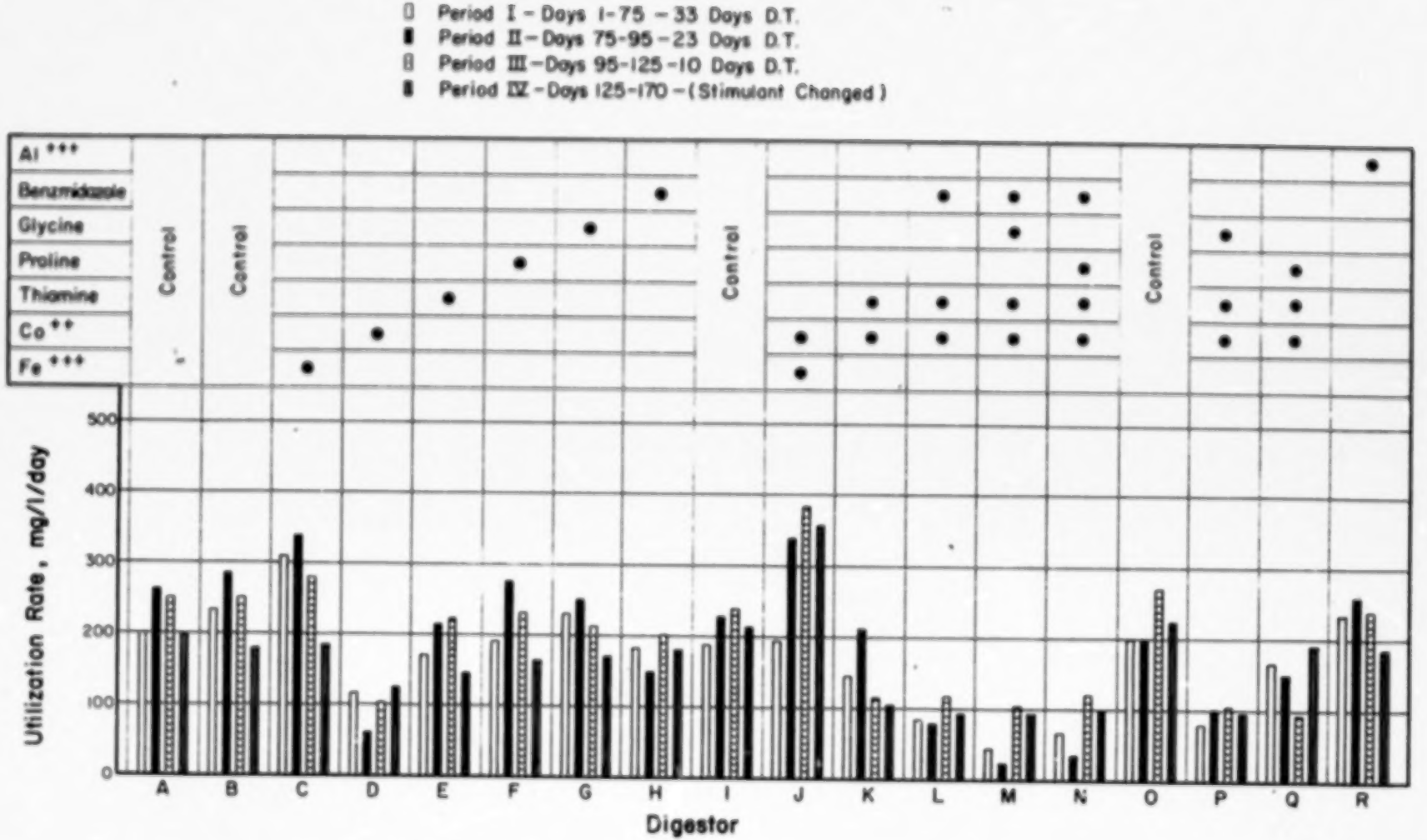

FIGURE 4. BAR GRAPH COMPARISON OF ADDITIVES.

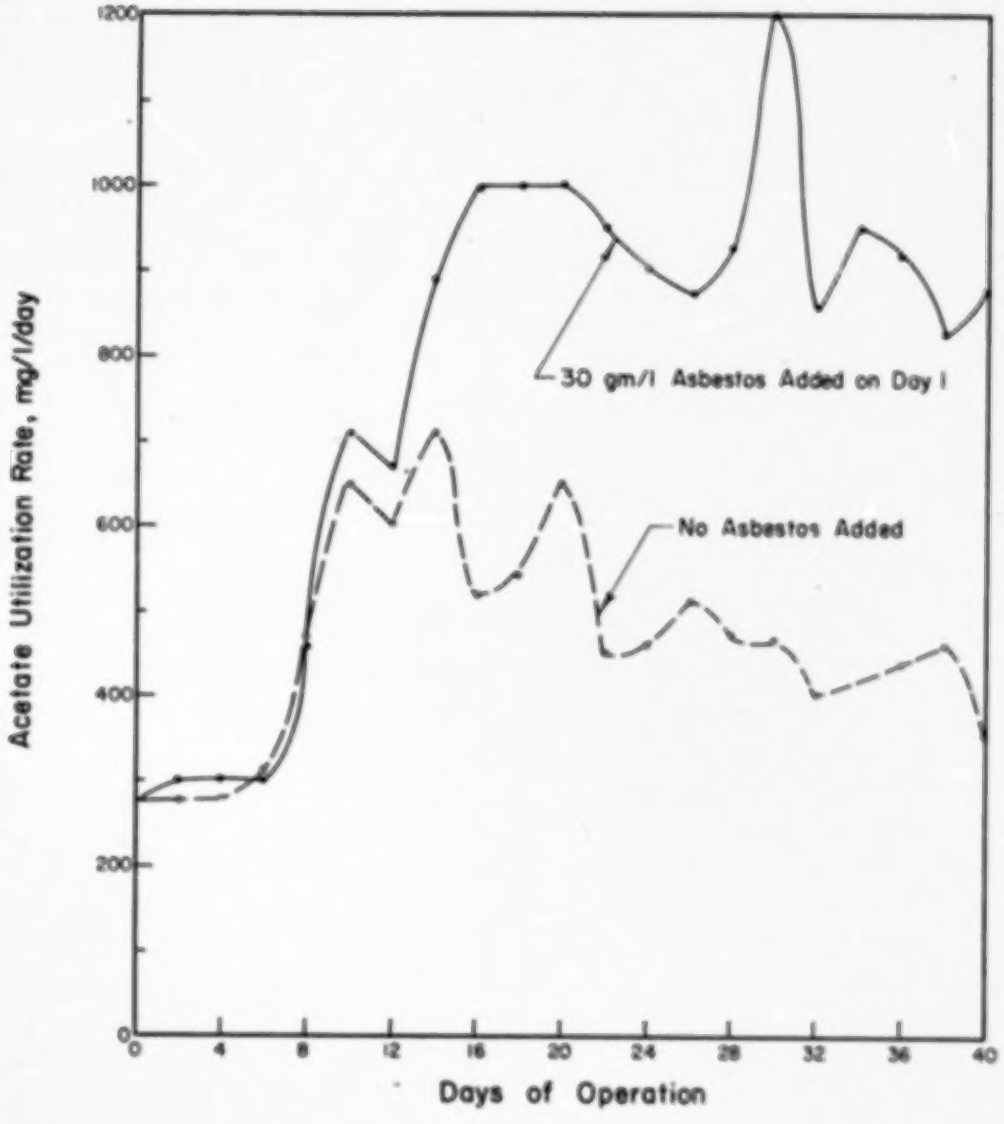

FIGURE 5. EFFECT OF ASBESTOS ON DIGESTION RATE.

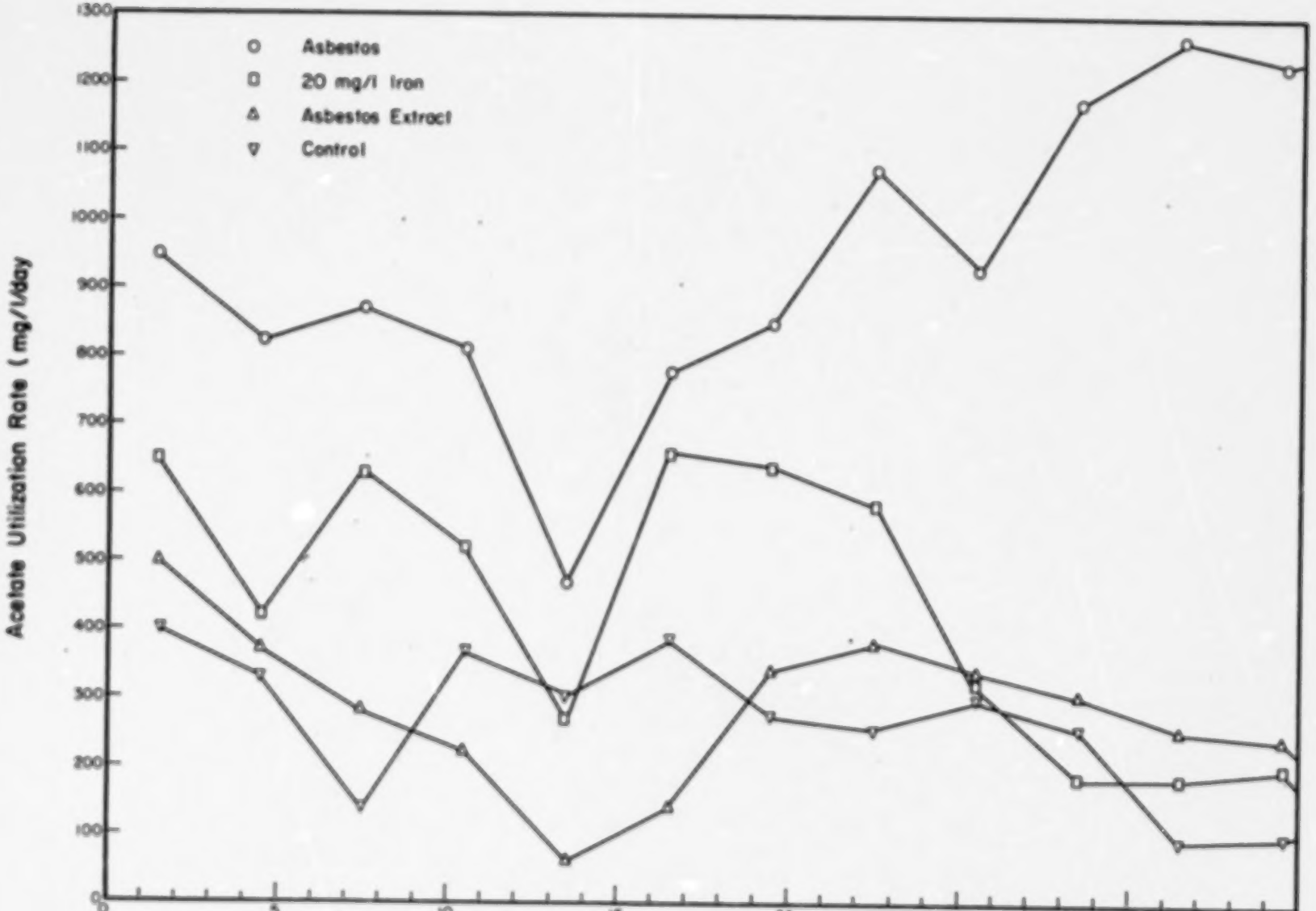

FIGURE 6. COMPARATIVE EFFECT OF ASBESTOS, ASBESTOS EXTRACT AND IRON ON DIGESTION RATE.

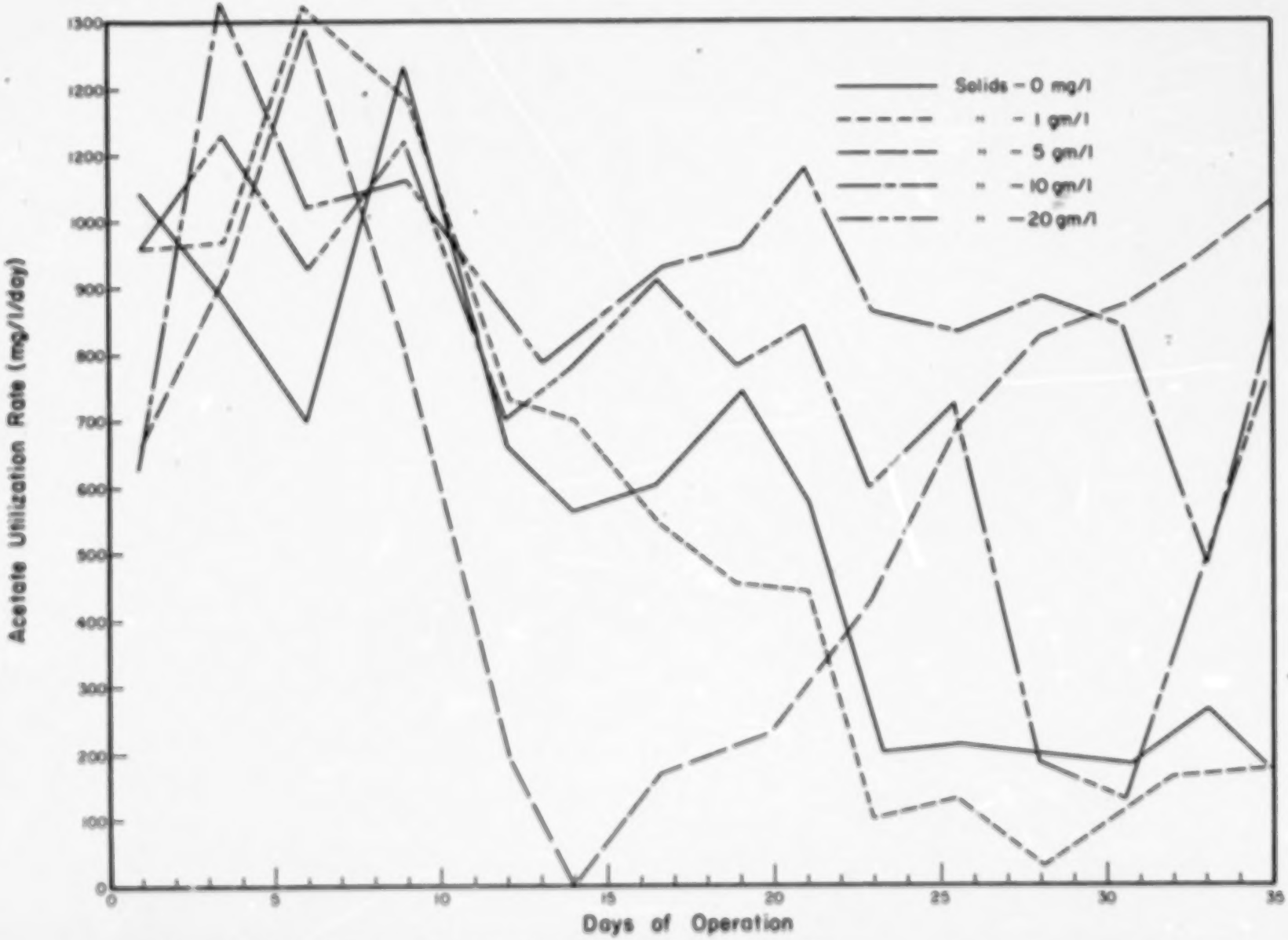

FIGURE 7. EFFECT OF SOLIDS ON DIGESTION RATE.

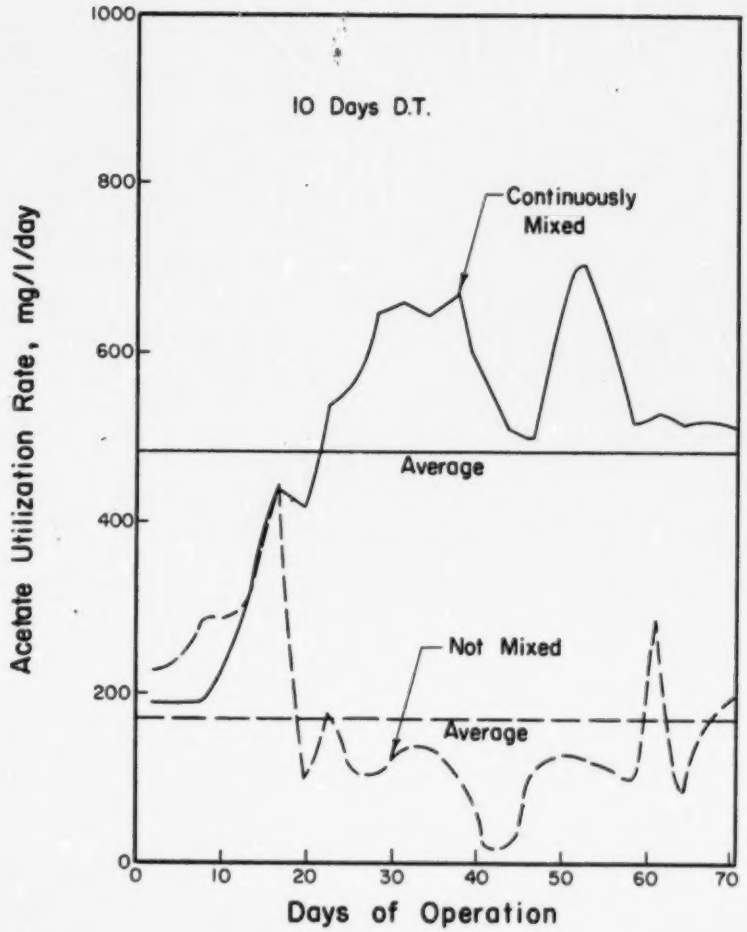

FIGURE 8. EFFECT OF MIXING ON DIGESTION RATE.

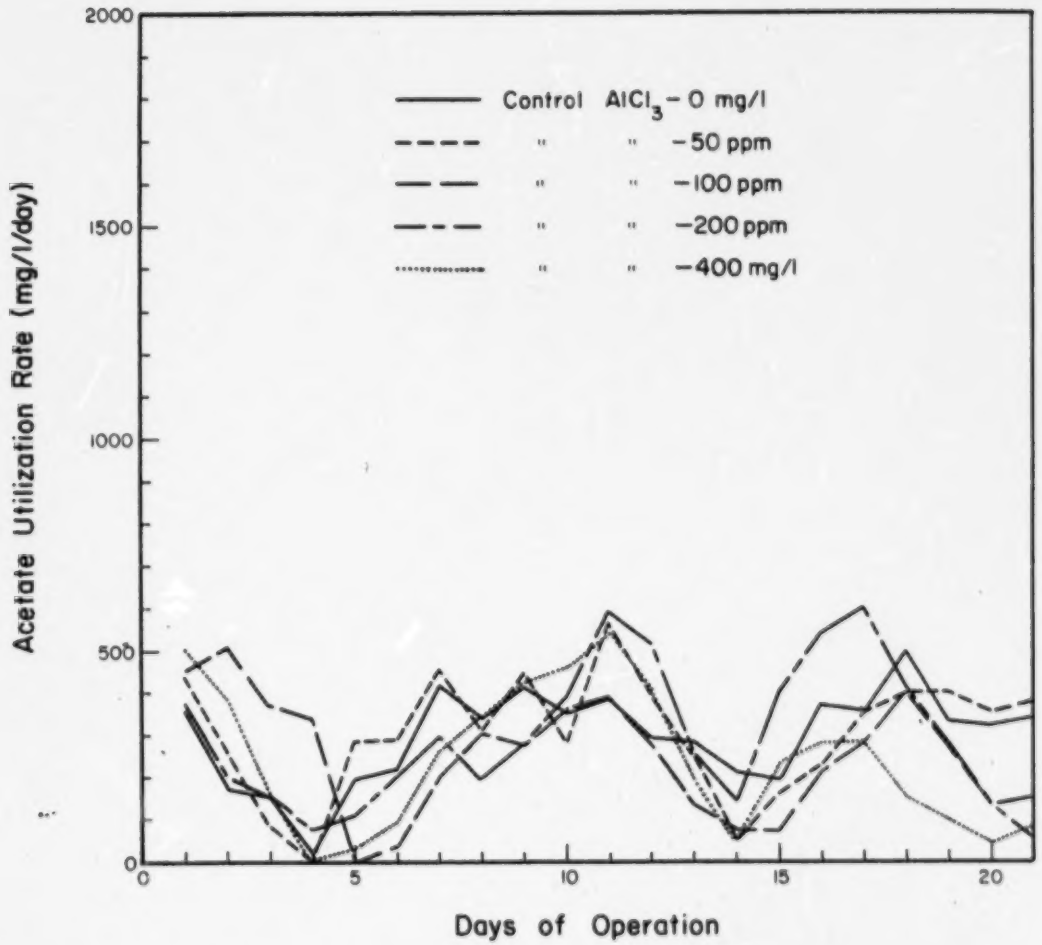

FIGURE 9. EFFECT OF COAGULATION ON DIGESTION RATE.

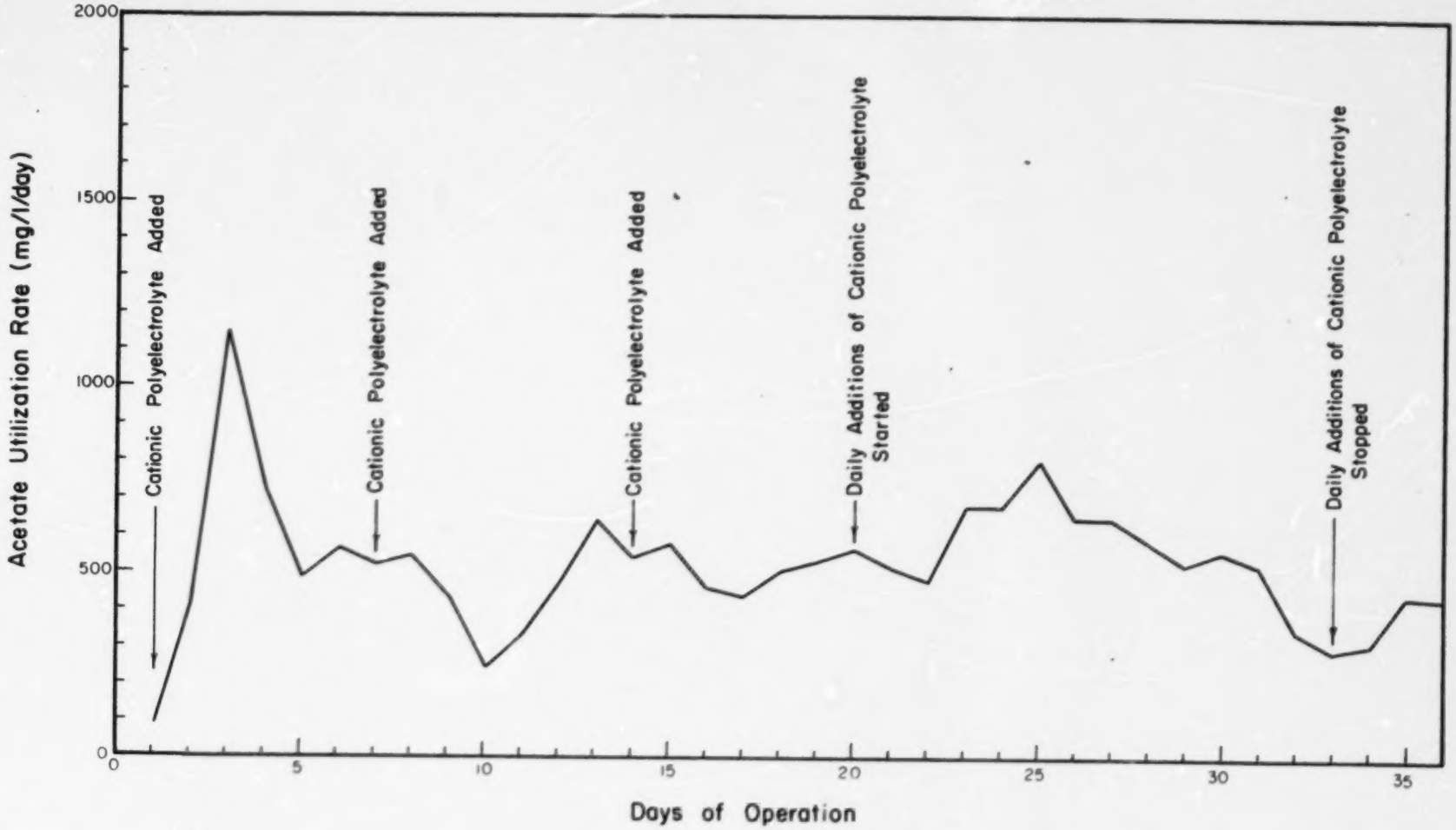

FIGURE 10. EFFECT OF CATIONIC POLYELECTROLYTE ON DIGESTION RATE.

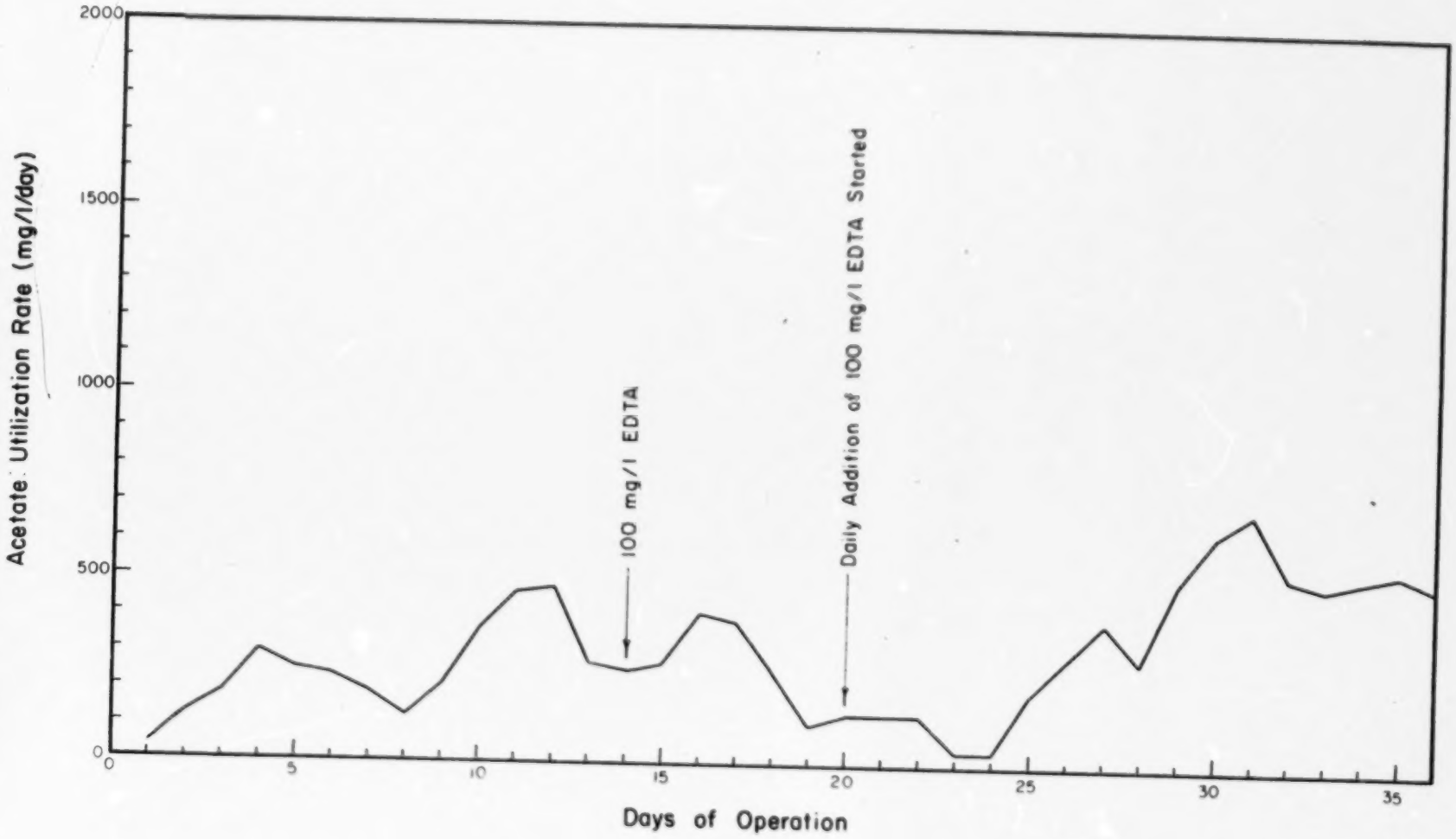

FIGURE 11. EFFECT OF COMPLEXING AGENT ON DIGESTION RATE.

THE EFFECT OF FREQUENT TEMPERATURE VARIATION ON METHANE PRODUCTION

Presented at the Annual Meeting of the Central States Water Pollution Control Federation, June 1964 at Urbana, Illinois.

Introduction

The methane-forming microorganisms are generally considered to be more sensitive to physical and chemical changes than the acid-forming microorganisms involved in the anaerobic digestion process. Also, because the methane-forming microorganisms utilize the volatile acid end-products produced by the acid-forming microorganisms, failure of the methane-formers to utilize the volatile-acids at approximately the same rate as they are produced, can result in a "stuck" digester. As a result, the fastidious nature of the methane-forming organisms combined with the critical position they occupy in the anaerobic digestion scheme, makes it very important that satisfactory environmental conditions be maintained for them in order to promote good digestion. However, in normal anaerobic digestion, the volatile acid concentration is low, indicating that the methane-forming organisms are capable of utilizing the volatile acids at least as fast as they are being formed. In other words, the acid formation is the rate-limiting step in normal digestion.

A number of studies have been made of the effect of temperature on the anaerobic digestion process (1), (2), (3). These studies have been made using a complex feed such as primary sludges. When using complex substrates, both the acid-formation and methane-formation rates are involved and the net overall effect being observed is actually controlled by whatever the rate-limiting step in the process happens to be. If the volatile acid concentration is low, then the acid-forming step determines the overall rate which is observed.

The objectives of this study are to determine the effect on the methane-forming organisms in a mesophilic sludge under the following conditions:

1. A temperature change sustained for a number of hours as would occur if the temperature of the digester contents rose or fell over a 24 hour interval.

2. A temperature drop for a 15 minute interval as would occur when digested sludge is pre-mixed with the raw sludge before it is pumped to the digester.

3. A temperature drop for a 2 hour interval as would occur if a proposed "anaerobic" contact stabilization process were feasible.

Presently, cold, diluted wastes are uneconomical to treat by anaerobic digestion due to excessive heat requirements. However, if the wastes could be adsorbed on a bacterial or inert surface and thus concentrated, it may prove economical to heat this more concentrated form of the sludge and stabilize it by anaerobic digestion. Such a system is shown schematically in Figure 1.

Procedure

A 2 l Erlenmeyer flask was placed in a temperature controlled bath as shown in Figure 2. The digester was purged with nitrogen gas and 1.8 l of sludge from a well operating 35° C digester was transferred to the flask. The digester contents were mixed by a magnetic stirrer placed underneath the bath. The temperature of the bath was controlled by a thermoregulator and it was kept in circulation by an air diffuser.

At the beginning of a run, the volatile acid concentration was determined. Sufficient acetate was then added to bring the volatile acid concentration to 700 to 1500 mg/l. Acetate was continuously fed to the digester with an electrolytic pump at approximately the same rate at which it was being utilized. Thus, feed concentration never limited the rate of gas production.

Since acetate is the predominate volatile acid present in a digester, this was used as the sole energy source. Since only the methane-formers can use acetate under anaerobic conditions, only the rate of activity of these organisms was observed.

At the start of a temperature drop study, the temperature was maintained for about an hour at the level to which it was to be raised after the drop. Gas production was recorded at 10-minute intervals during this first hour, during the drop, and for a sufficient period after the drop to reach equilibrium. These readings were continued during the temperature drop.

Test runs were made to observe the effects of both temperature increases and decreases on the rate of gas production. Observations were made on gas production for the following temperature schedules:

Initial Temperature (C°)	Drop Temperature (C°)	Duration of Temperature Drop (Min)
35	10	15
35	10	120
35	20	15
35	20	120
50	10	120

Feeding calcium acetate as the sole substrate results in a gas composition of 75% methane and 25% carbon dioxide. The CO_2 introduces an error into the gas production values with each temperature change, however, due to its high solubility in water and the great dependence of CO_2 solubility on temperature. As a result, following each temperature increase, there is an initial marked increase in gas production, after which gas production comes to an equilibrium at a somewhat lower rate. This spike in the gas production is accounted for by the decreased solubility of CO_2 at higher temperatues and therefore the release of CO_2 from solution. A correction was not made because the exact percentage of CO_2 in the digester atmosphere

would have to be known both during the temperature drop when CO_2 would be somewhat above the theoretical 25%. The CO_2 equilibrium of the digester contents and atmosphere was established soon after a temperature increase occured because the volume at the top of the flask was small and it was rapidly flushed out by the gas evolved.

Figure 3 shows the clear response of gas production from mesophilic sludge which had been incubated at 35°C and was incubated for a number of hours at the temperature shown. This figure indicates gas production bears a linear response to temperature as opposed to the traditional logarithmic response. Below a temperature threshold of 20° C, 68° F, gas production was insignificant for the periods observed. This observation is also borne out by later figures.

From this figure, it is seen that a decrease in digester temperature from 37°C to 30°C reduces the gas production to approximately 50%. Golueke (1) found that the destruction of volatile matter decreased from 50% to 40% when the digestion temperature was decreased from the range of 35 - 55°C down to 30°C. Thus, while volatile matter destruction at 30°C decreased to only 80% of that at 37°C, gas production would suffer much more severely by decreasing to 50% of the original.

As shown in Figure 4, the response of the methane-formers to a temperature change is immediate. As the temperature was dropped over an hour's period of time from 34°C to 27°C, the gas production decreased to about 12% of that at 34°C. As the temperature was increased from 27°C back to 33°C, the gas production resumed at approximately 100% of that before the temperature drop occurred. A subsequent increase in temperature to 40°C was accompanied by gas production rates of about 140% or those at 34°C.

Figures 5 through 8 indicate that as long as the digester temperature is below 20°C, gas production is insignificant for the periods shown. However, as soon as the temperature is raised back to the normal range for anaerobic digestion, gas production resumes at a rate proportional to the temperature within the range 20 to 45°C. There appears to be no carryover of the adverse effect on the methane-formers from exposure to temperatures of 10 and 20°C for 15 minutes. Gas production resumed at essentially the same rate as the temperature was restored to the initial level.

Figure 6 shows a slight lag in recovery of gas production after maintaining a sludge at 10°C for two hours. It is noted that temperature recovery was also slow in this case due to the type of water bath used during this run. This equipment was modified in later experiments to allow more rapid temperature recovery.

Figures 7 and 8 show that after equilibrium conditions have been established, about 20% of the initial gas production occurred at 20°C and, again, there was no lag apparent except for the lack of a "spike" due to CO_2 release.

Figure 9 shows no gas production lag for a sludge held at 10°C for 2 hours and then raised to 50°C. In conclusion to this portion of the experiment, the methane-forming organisms appear to be very adaptable to frequent temperature changes with no adverse effects resulting from temperature drops within the ranges studied.

It was desirable in the course of this study to confirm the effect of temperature on both the acid-formation rate and gas production rate. To accomplish this, an actively digesting sample of sludge was taken from the primary digester at the Champaign-Urbana Sewage Treatment Plant. This insured that both groups of microorganisms were functioning well. An increase in volatile acids concentration was simulated by adding sufficient acetate to raise the volatile acids concentration of the sludge to 1800 mg/l, followed by 10% by volume of primary sludge to insure adequate substrate for the acid-forming organisms. The sludge was then divided into 2 portions and placed in flasks which had been purged of oxygen by flushing with nitrogen and carbon dioxide gas.

The contents of one flask were maintained at 35°C and the second at 45°C. Both flasks were continually mixed and once a day 1/20 of the contents were withdrawn and replaced by an equal amount of raw sludge. The raw sludge feed simulated normal digester operation and provided a food source for the acid forming microorganisms, and the volatile acids provided a food source for the methane-forming organisms. Thus, by comparing the volatile acids concentration and gas production in the 45°C digester with that in the 35°C digester, the effect of a temperature increase on the relative rates of activity of the two groups of organisms, the acid-formers and the methane-formers, could be determined.

If the activity of both groups of microorganisms was dependent to the same degree of temperature, the volatile acids concentration in both the 35 and 45°C digesters should be equal, because the increased volatile acid utilization would be matched by a corresponding increased volatile acid formation. If the volatile acids concentration was lower in the 45°C digester than in the 35°C digester, this would indicate that the activity of the methane-formers increased at a greater rate with temperature than the acid-formers.

Figure 10 reveals a very significant comparison. It shows the beneficial effect of increased temperature on a digester in which the methane-formers are not inhibited by anything but temperature; that is, there are no adverse environmental or physiological conditions restricting the rate of activity of the methane-formers except temperature. In less than 30 hours, the volatile acid concentration in the 45°C digester had dropped to the original level of 300 mg/l. However, in the 35°C digester the volatile acids concentration had dropped only to about 1400 mg/l.

It would be expected that the increase in temperature would not only increase the rate of activity of the methane-formers, but also the rate of activity of the acid-formers. This is borne out by calculation on the data from each 2 liter digester. At 38.5 hours, 4000 ml of gas was produced by the 45°C digester and the volatile acids had decreased by 2.92 grams in the 2 liter digester. Assuming 600 ml of gas is produced per gram of volatile acids destroyed, then 4000 ml of gas represents the destruction of 6.67 grams of volatile acids. Since the amount of volatile acids in the digester was only reduced by 2.92 grams, then the remaining 3.75 grams of volatile acids must have been contributed by the acid-formers during this time. By simular calculations, it is found that only 1.64 grams of volatile acids were contributed by the acid-formers in the 35°C digester in the same time.

It is concluded from Figure 10 that while an increase in temperature does increase the activity of both the methane-forming organisms and the acid-formers, the methane-formers increase their activity at a considerably greater rate per degree. Thus, not only can the methane-formers consume the additional volatile acids contributed by the acid-formers at higher temperatures, but in addition, they are able to reduce the pool of volatile acids which is initially present. This explains the rapid decrease in volatile acids in the 45°C digester as compared to the less rapid decrease in the 35°C digester.

Engineering Significance

Figure 11 is hypothesized as a characteristic plot of the temperature dependence of the acid-forming and methane-forming microorganisms in a favorable anaerobic digestion environment. Above a certain temperature, X, the methane-formers are capable of consuming volatile acids at a rate greater than the rate at which they are supplied by the acid-formers. Therefore, the volatile acids concentration remains low. Below this temperature, X, the rate of activity of the methane-formers is lower than the acid-formers. The net result is that volatile acids start to accumulate in the digester.

Experience seems to indicate that the temperature, X, at which the rates are equal is approximately 30 to 35°C. There appears to be only a minor advantage to be gained by maintaining the temperature of a sewage sludge digester above this range, since the rate of liquefaction is not appreciably increased, according to Golueke (1), and Heukelekian and Kaplovsky (2). The results, as shown in Figure 10, indicate a definite rate increase in acid-formation but not as much as the rate increase in the methane formation. Above temperature, X, acid formation is the lower and thus the rate-limiting step in the two-step digestion process, and the increased potential capacity of the methane-formers cannot be exploited because they have only a limited food supply.

In the anaerobic digestion of a soluble industrial waste, the rate of acid formation may be appreciably greater than with a complex solid, like sewage sludge. In such a case, advantage could be taken of the markedly greater rate of methane formation at the 40 to 45°C level as compared to the 35°C commonly used. This would result in a much smaller digester.

Pre-mixing of digested sludge with raw sludge before pumping the mixture into the digester appears to stop methane production if the temperature of the mixture would be lowered to less than 20°C. However, no retardation of gas production results when the temperature is restored to normal inside the digester.

On this basis, the flow diagram proposed in Figure 1 appears to be feasible with respect to the methane-forming organisms in that they are able to adapt to the changing temperatures. The adsorptive concentration of the pollutant would be cirtical and require considerable study.

Thus study was supported by Grant WP-00394 from the Division of Water Supply and Pollution Control United States Public Health Service.

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Sewage and Industrial Wastes 30, p. 1225, (1958)

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Plant-Scale Studies of Thermophilic Digestion at Los Angeles
Sewage and Industrial Wastes 26, p. 1202 (1954)

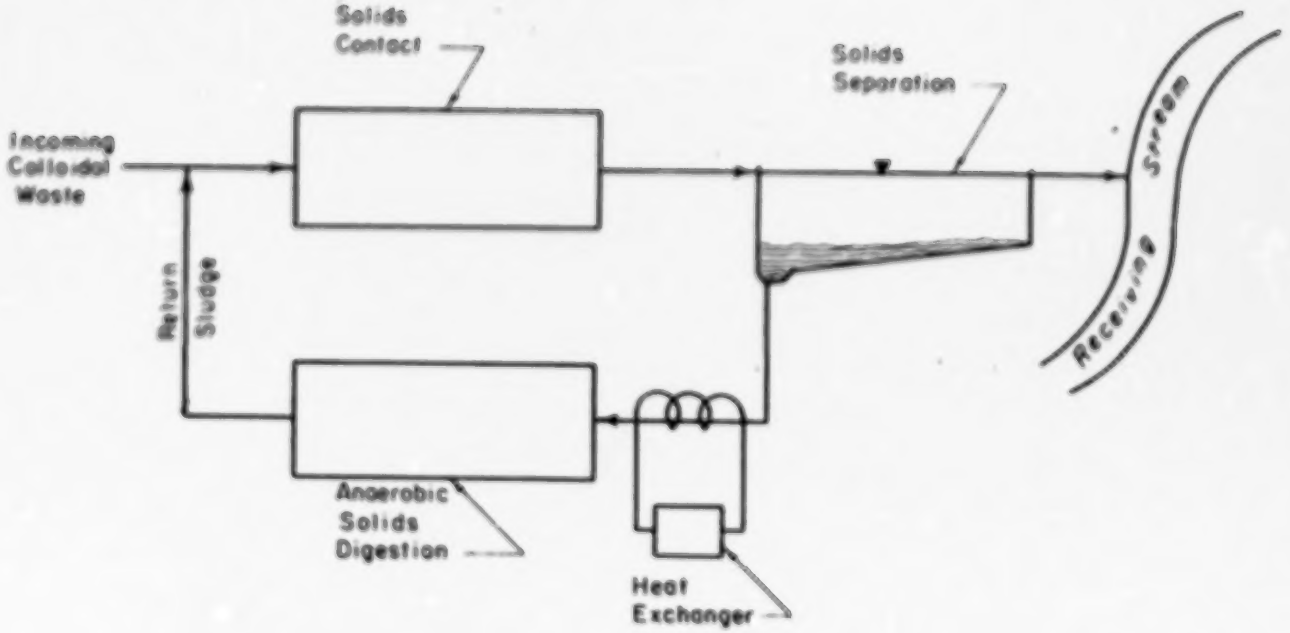

FIGURE 1. PROPOSED ANAEROBIC CONTACT STABILIZATION PROCESS.

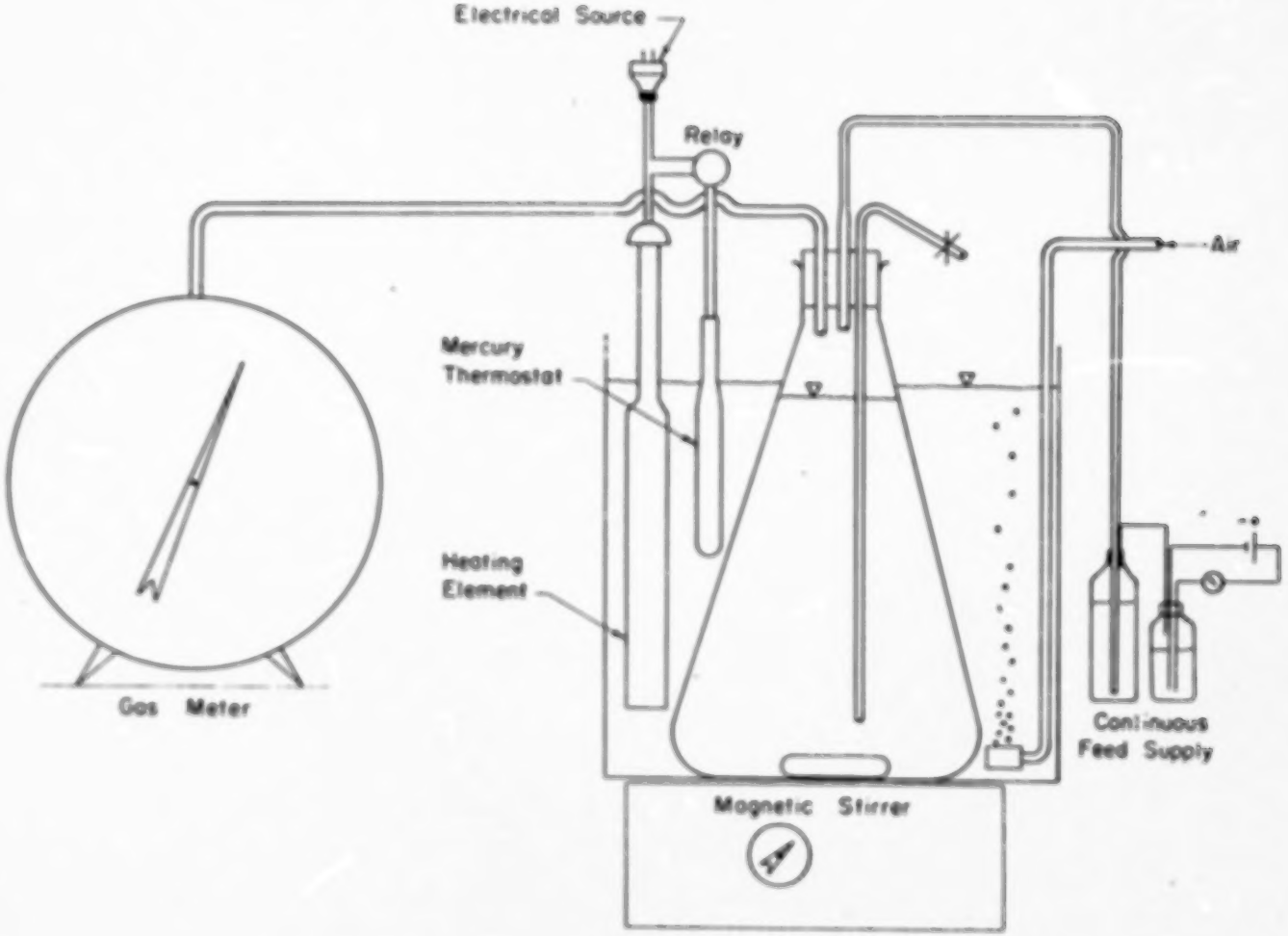

FIGURE 2. SCHEMATIC DIGESTION APPARATUS.

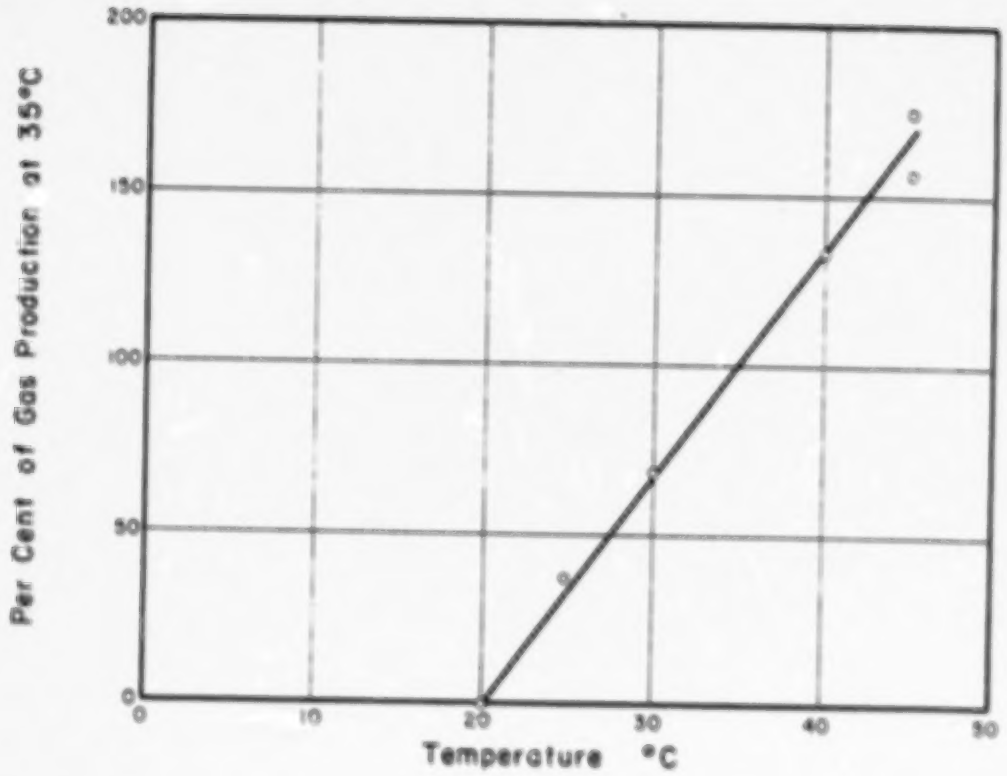

FIGURE 3. RELATIVE GAS PRODUCTION vs TEMPERATURE .

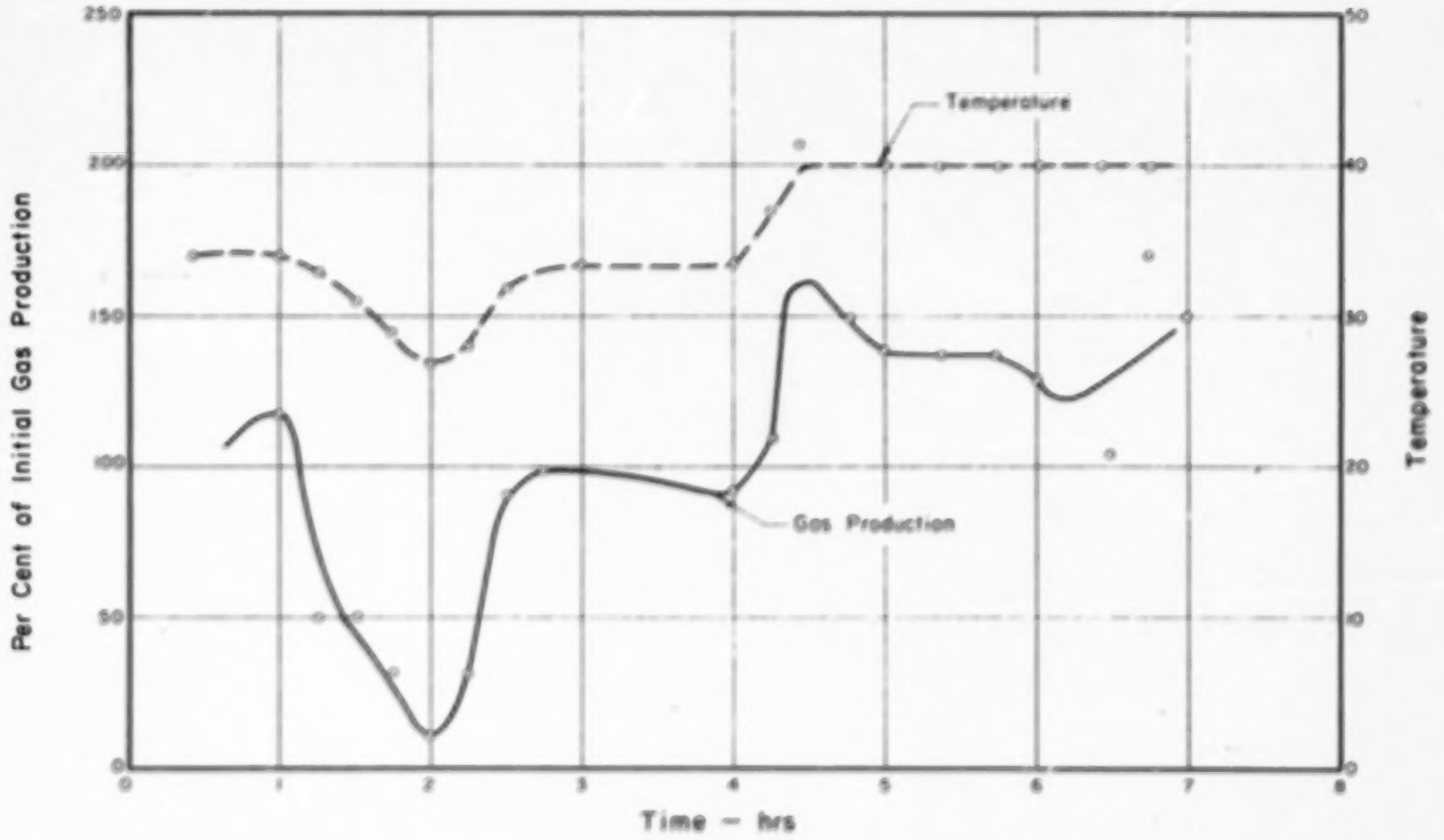

FIGURE 4. DEPENDENCE OF GAS PRODUCTION ON TEMPERATURE.

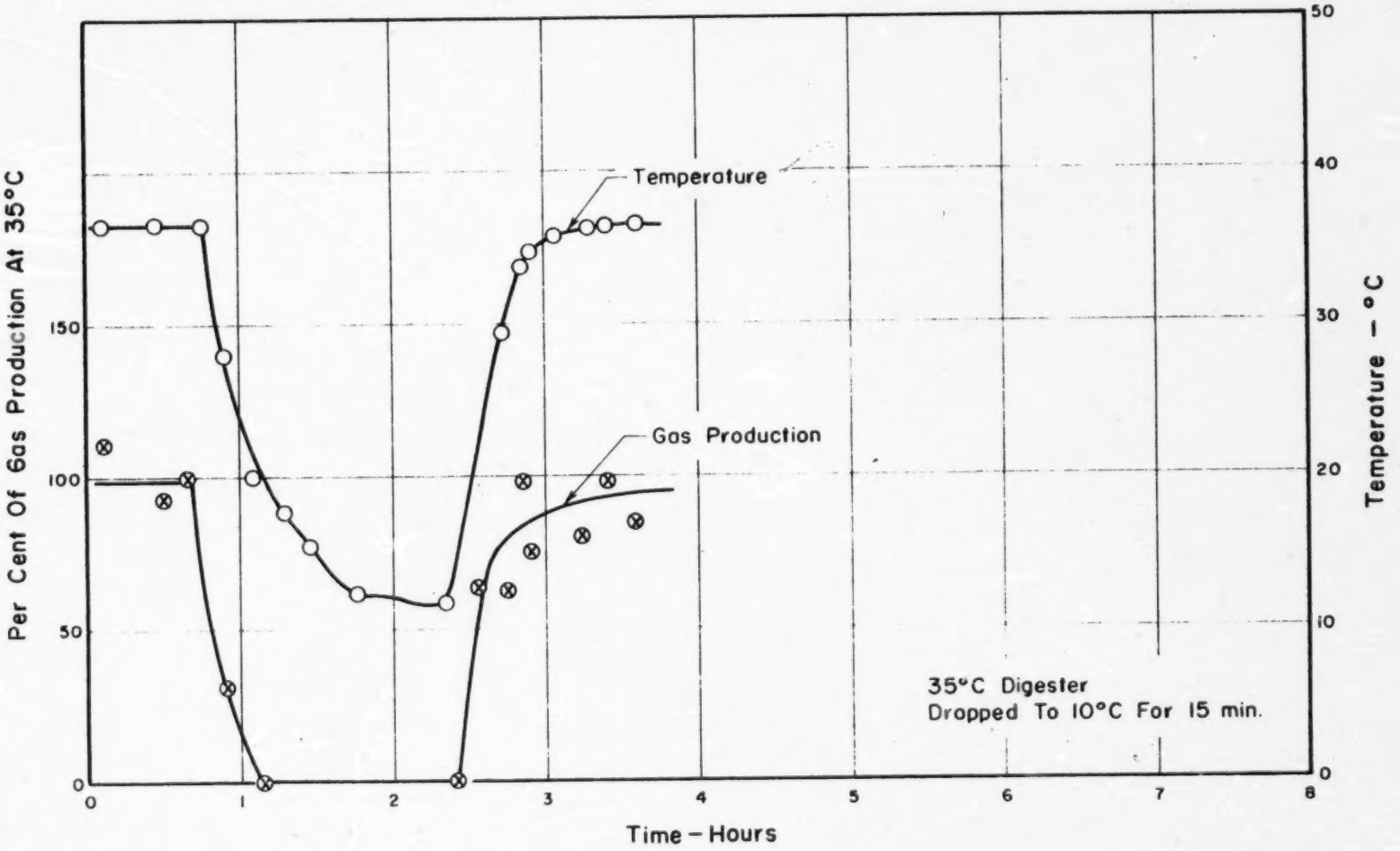

FIGURE 5. GAS PRODUCTION VS TEMPERATURE.

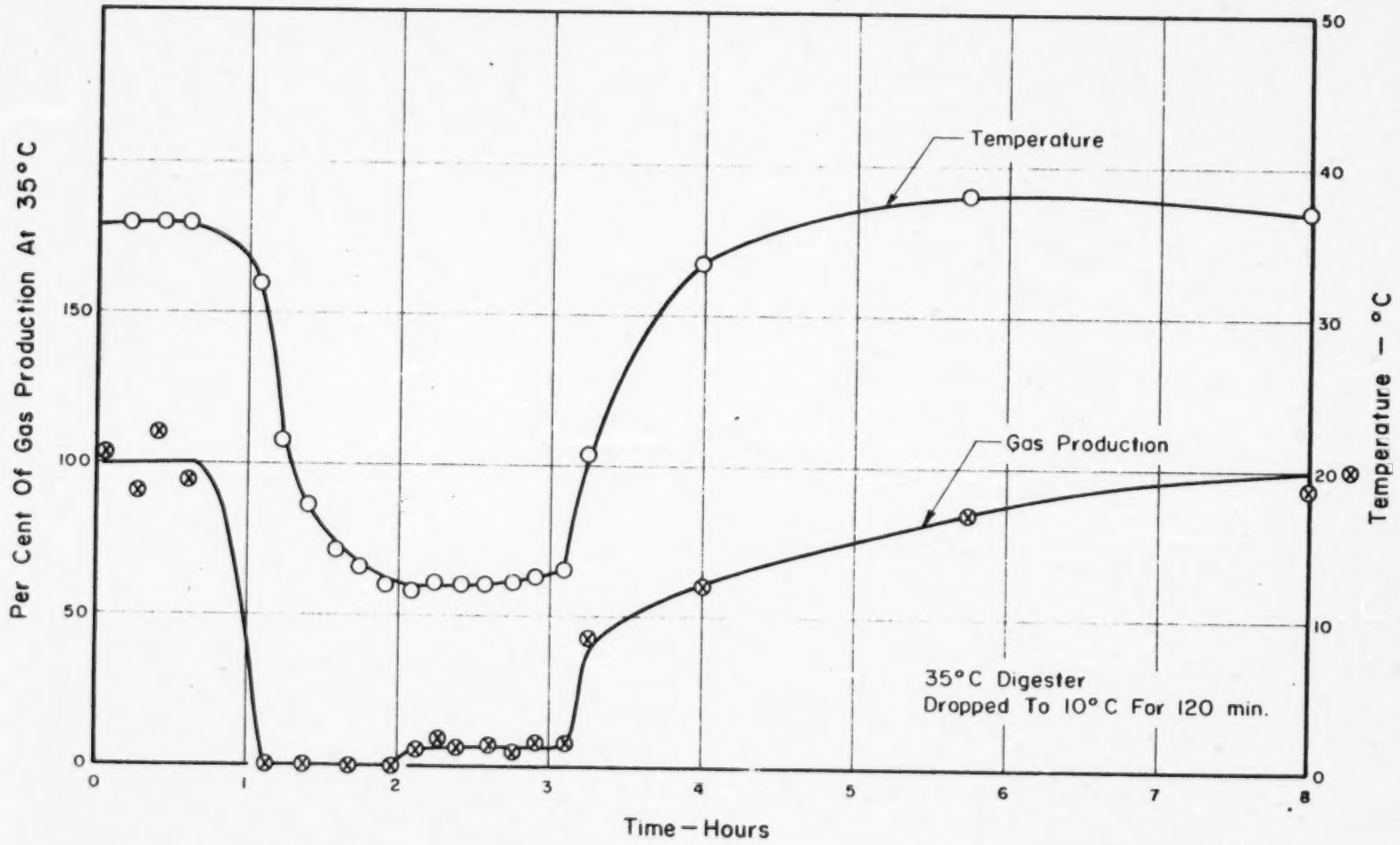

FIGURE 6. GAS PRODUCTION VS TEMPERATURE.

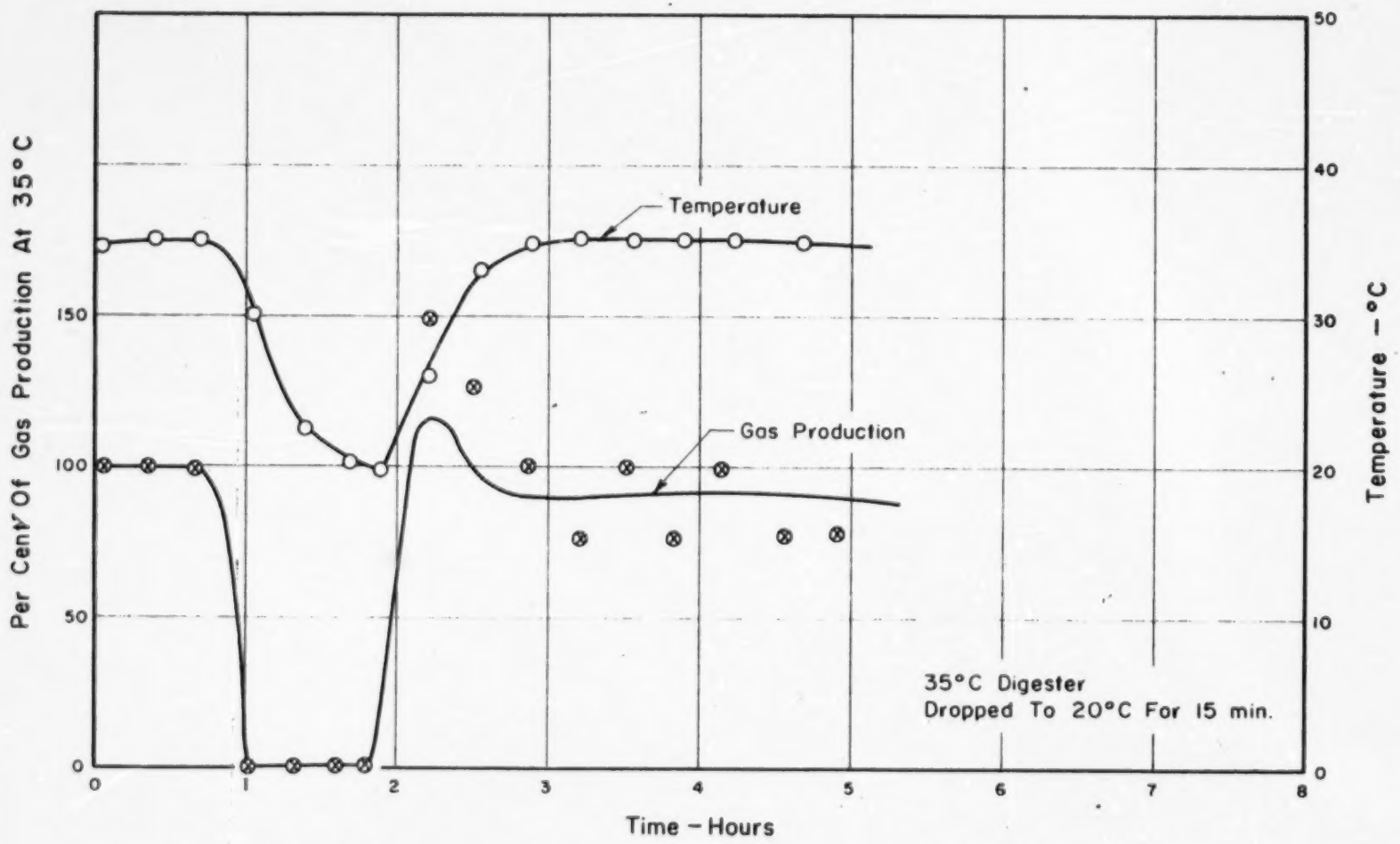

FIGURE 7. GAS PRODUCTION VS TEMPERATURE.

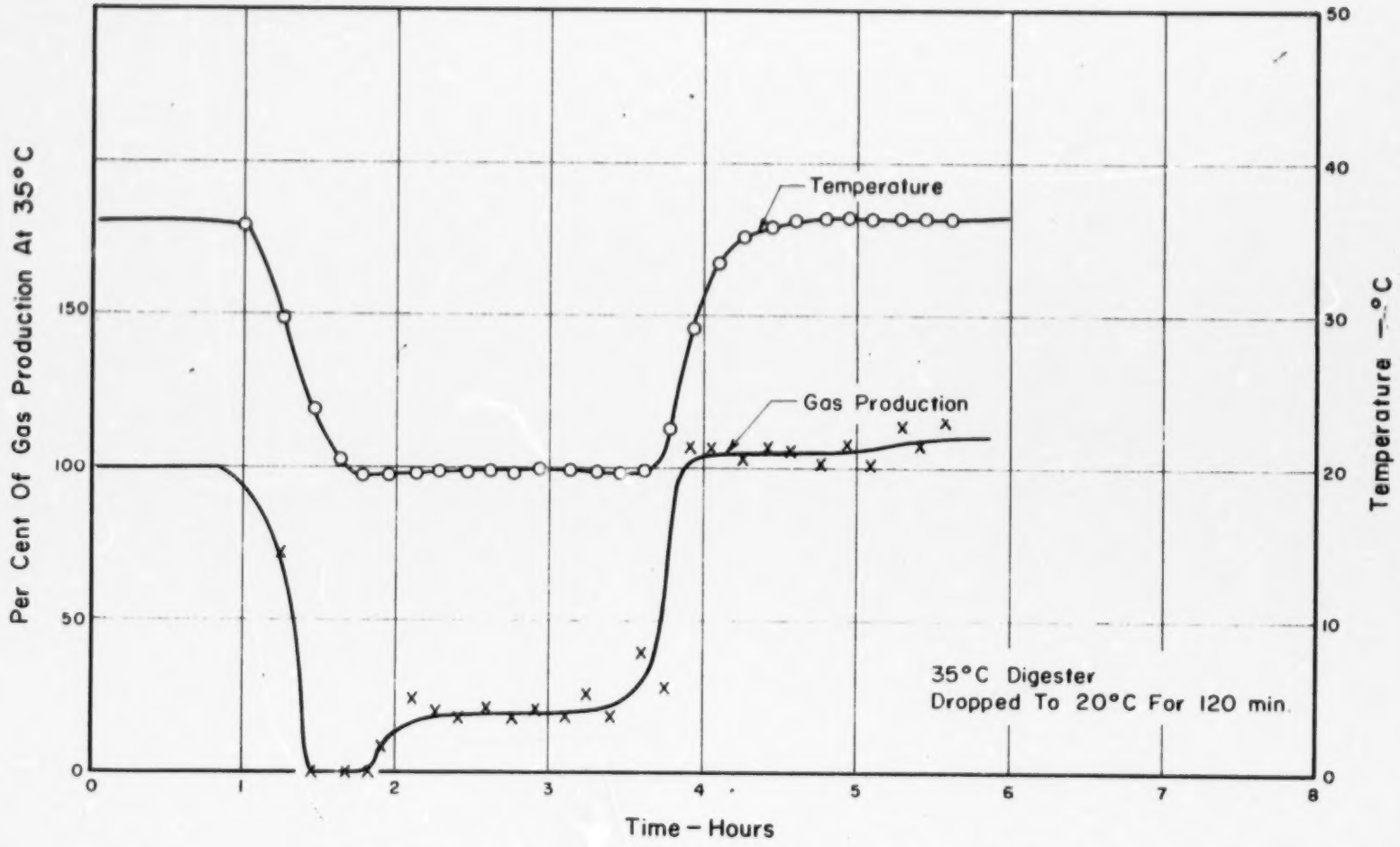

FIGURE 8. GAS PRODUCTION VS TEMPERATURE.

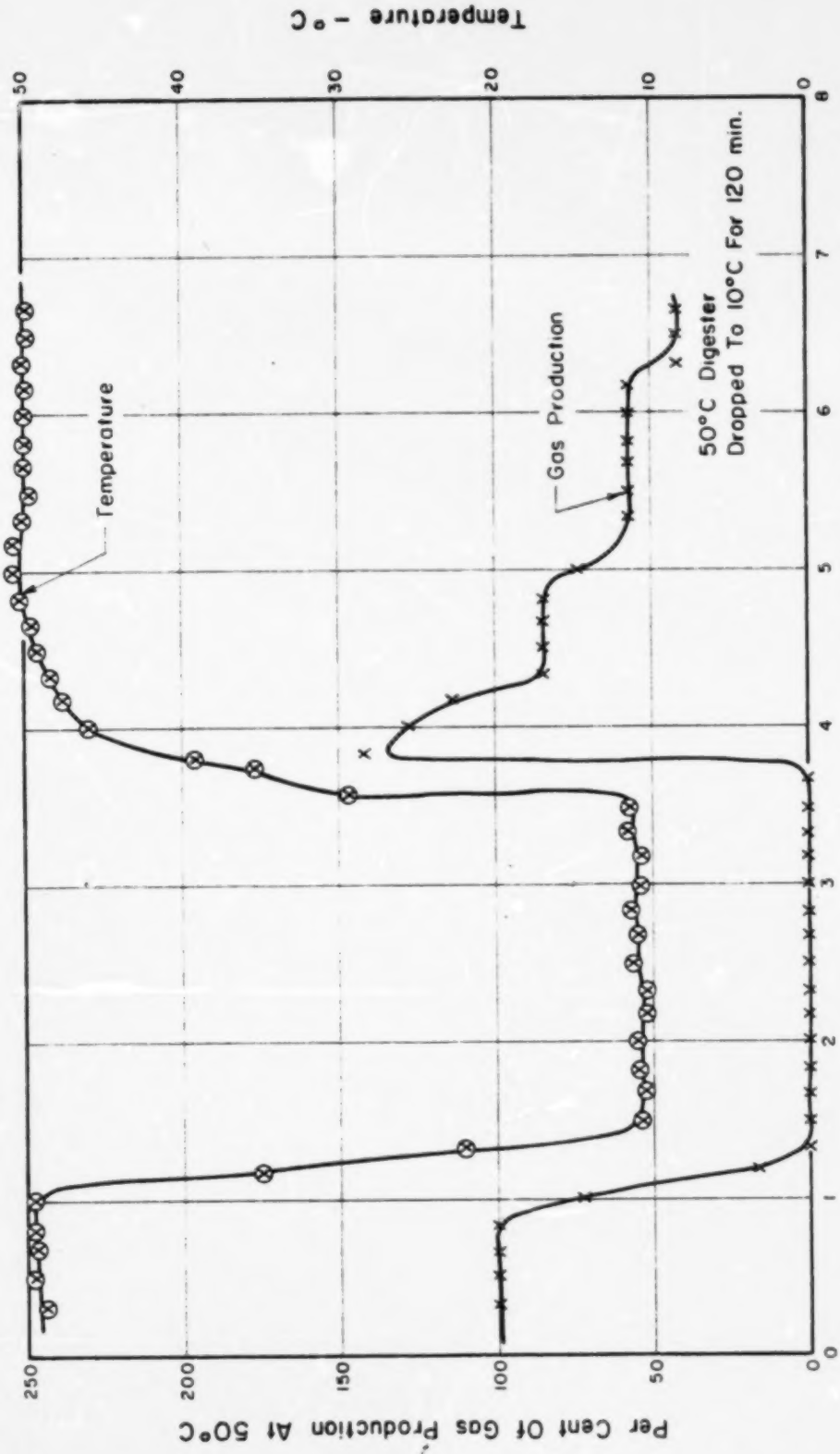

FIGURE 9. GAS PRODUCTION VS TEMPERATURE.

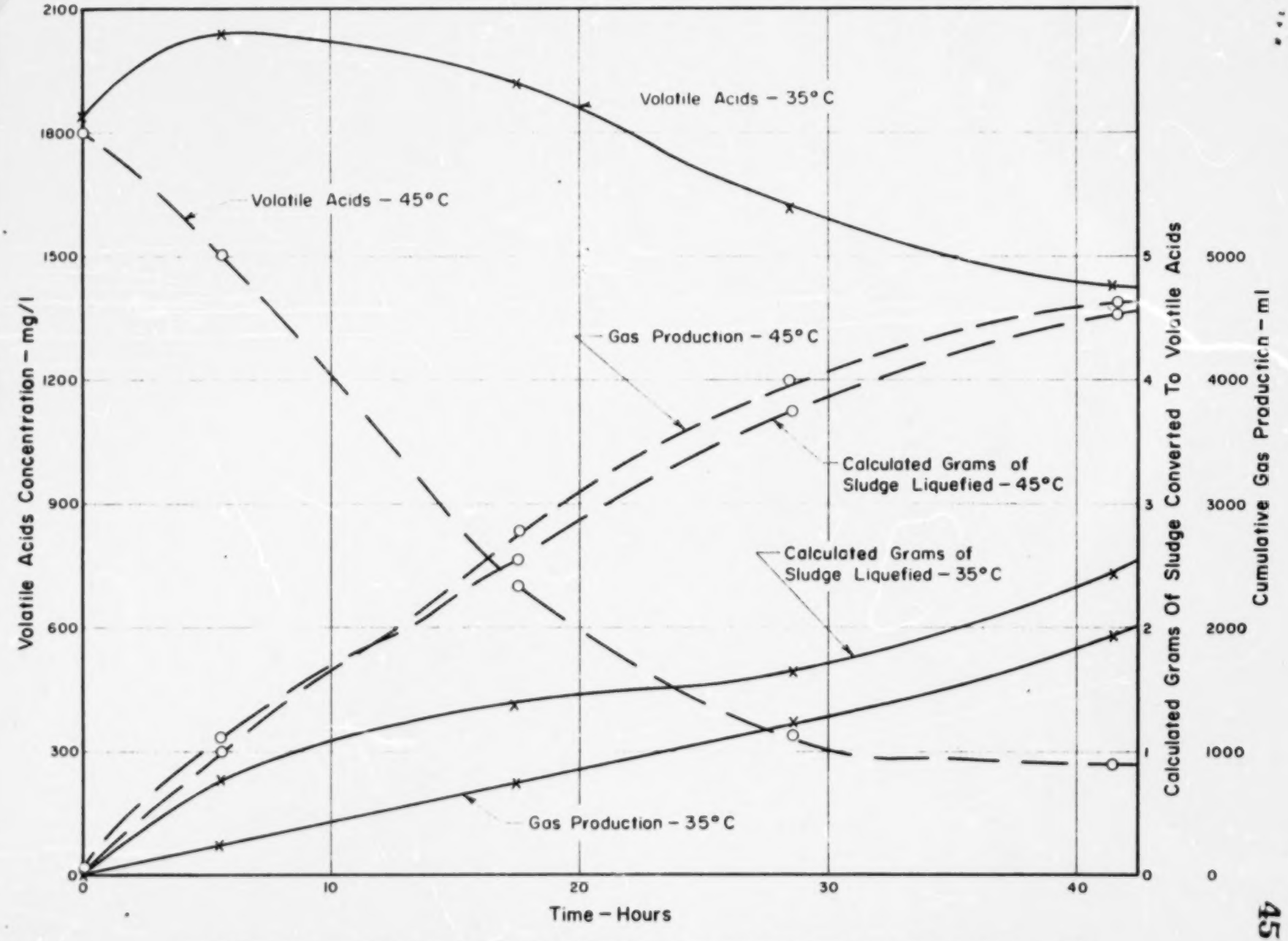

FIGURE 10. COMPARATIVE DIGESTER PERFORMANCE AT 35°C AND 45°C.

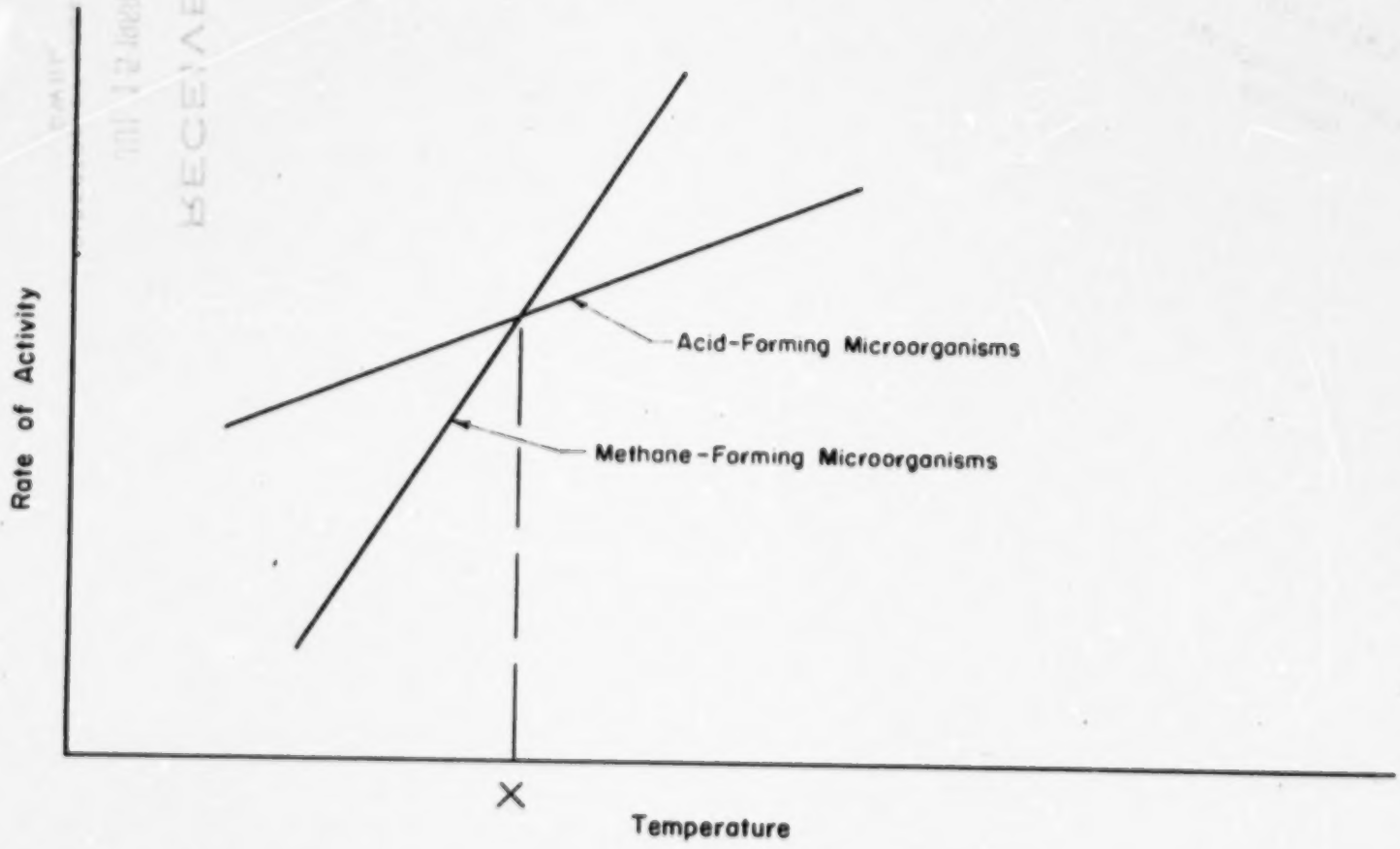

FIGURE 11. TEMPERATURE DEPENDENCE OF ACID-FORMING AND METHANE-FORMING MICROORGANISMS IN ANAEROBIC DIGESTION OF SEWAGE SLUDGE.